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Original article

Acute and sub-chronic toxicity studies of a standardized extract of *Centella asiatica* ECa 233

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Abstract:

Acute toxicity study of *Centella asiatica* standardized extract ECa 233 was conducted by an oral administration of 10.0 g/kg extract into 10 male and 10 female mice. The extract at the given dose did not cause any toxic signs and death within the observation period of 14 days. Sub-chronic toxicity study of ECa 233 has been investigated in four groups of Wistar rats, each of 24 rats (12 of each sex). Control group was orally given distilled water and three experimental groups were orally administered with ECa 233 in distilled water at the doses of 10, 100, and 1,000 mg/kg/day for 90 days. All ECa 233-treated rats showed no difference with regards to body weight, food consumption and health in comparison to the control group except that female rats receiving 1,000 mg/kg/day of ECa 233 had significantly higher white blood cell counts than the control group ($p < 0.05$). However, this alteration was not associated with internal organ pathology. In addition, male rats receiving 1,000 mg/kg/day of ECa 233 had significantly higher sodium level, but still within normal range, than those of control group ($p < 0.05$). Histopathological results of internal organs did not demonstrate any incidence or degree of lesions in a dose-dependent manner with the increasing dose of ECa 233. Therefore, based on the present studies it can be concluded that ECa 233 in the dose up to 10.0 g/kg produced no acute toxicity and no significant sub-chronic toxicity was observed in rats receiving 10-1,000 mg/kg of ECa 233.

Keywords: Toxicity; ECa 233; Standardized extract of *Centella asiatica*

Introduction

Centella asiatica (Linn.) or Indian Pennywort is a perennial plant that has been used for many medicinal purposes worldwide since ancient time. In Ayurveda medicine, *C. asiatica* was described as one of neuronutrient which could prolong life and enhanced memory [1]. Pharmacological evaluation conducted in animal models has demonstrated a variety of pharmacological effects such as anti-inflammation, anti-oxidation, wound healing, anti-infective and anti-dementia [2]. In Thailand, in addition to traditional medicinal benefit such as brain and heart tonic or wound healing effect, whole plant of *C. asiatica* is regarded as a vegetable and its juice is consumed as a beverage. Except for few reports on contact dermatitis, no serious adverse effect of *C. asiatica* has been recognized [3]. Safety of consumption of dried plant has been proved in toxicity testing in which the median lethal dose of dried powder of *C. asiatica*, given orally into mice, was found to be higher than 8 g/kg. In chronic toxicity study, Wistar rats of both sex receiving 20, 200, 600 and 1,200 mg/kg/day of *C. asiatica* for 6 months showed no sign of significant alteration of body weight, blood chemistry, clinical chemistry or histopathology in comparison to control group [4]. In contrast, hepatic damage was reported in albino rats receiving oral administration of dried *C. asiatica* at the dose of 1,000 mg/kg/day for 30 days [5]. ECa 233 is the standardized extract of *C. asiatica* defined as a white to off white titrated extract of *C. asiatica* containing not less than 80% of triterpenoids and the ratio between madecassocide and asiaticoside should be within 1.5 ± 0.5. Preliminary study on its pharmacological activity demonstrated ameliorating effects of ECa233 on learning and memory deficit induced by an intracerebroventricular injection of β-amyloid peptide (25-35) into rats [6]. In addition, topically applied gel containing 0.05% ECa233 on second degree burn wound was found to significantly increase blood flow and subsequently accelerate rate of wound healing in rats [7]. However, except for the report of favorable profile of ECa 233 on rat hepatic cytochrome P450 [8], no other information of its safety is available. Therefore, we consider it interesting to conduct acute as well as sub-chronic toxicity study of ECa 233.

Materials and Methods

Standardized extract of C. asiatica ECa 233

ECa 233 was kindly supplied by Associate Professor Ekarin Saifah, Ph.D. and Associate Professor Rutt Suttisri, Ph.D., Department of Pharmaceutical Botany. Quality control was made by Associate Professor Suwanna Luangcholatan and Assistant Professor Chamnan Patarapanch, Ph.D., Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. Various concentrations of ECa 233 were freshly prepared as a suspension in distilled water and being given orally to the animals by a gavage tubing. Care was taken to assure the homogenicity of the suspension before an administration into the animals.

Experimental animals

Twenty ICR mice, ten male and ten female, weighing between 20 to 22 g were used in acute toxicity test. Forty eight male and forty eight female Wistar rats weighing between 180 to 220 g were used in sub-chronic toxicity study. They were purchased from the National Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. Animals were housed in the conventional hygeinic animal room of Laboratory Animal Center, Department of Medical Sciences, Ministry of Public Health, Thailand. The room temperature was maintained at 25 ± 1 °C with 12-hour alternate light-dark cycle and 60% relative humidity. The mice were provided with commercial pellet (C.P. Company, Thailand) and water *ad libitum*. They were fasted for two hours before performing acute toxicity test. The animals were acclimatized for at least seven days prior to the experiments. The study protocol was approved by the Institutional Animal Care and Use Committee, Department of Medical Sciences, Ministry of Public Health, Thailand (permission no. 49-005).

Acute toxicity study

Both male and female mice were randomly divided

into two groups of ten mice each. The experimental group was orally administered with ECa 233 suspension at the dose of 10 g/kg. The animals were closely observed during the first three hours and then were observed twice daily for 14 consecutive days. At day 15, mice were euthanatized using CO_2 gas chamber and then necropsy was performed for inspection of gross pathological alterations.

Sub-chronic toxicity study

Both male and female rats were randomly divided into 4 groups of 12 rats each. Group 1 was a control group being given orally with 10 ml/kg of distilled water. Group 2 to 4 were treatment groups that were orally administered with ECa 233 at the doses of 10, 100 and 1,000 mg/kg/day for 90 consecutive days. The lowest dose (10 mg/kg/day) was the effective dose in attenuation of learning and memory deficit induced by an intracerebroventricular injection of β-amyloid peptide in rodent. During the period of experiment, body weight and food consumption were measured weekly and the animals were closely observed for general appearance, behavior and signs of abnormality. At the end of treatment, the animals were fasted for 16 hours before being sacrificed with diethyl ether inhalation. Blood samples were collected from the inferior vena cava of each animal for hematology and clinical chemistry determination using automatic hematological analyzer Cell Dyn® 3500 and Hitachi® 912, respectively. Hematological parameters examined were hematocrit (Hct), erythrocyte (RBC), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell (WBC), neutrophils, eosinophils, lymphocytes, monocytes, basophils and platelets. Clinical chemistry parameters measured were alkaline phosphatase (ALP), alanine aminotransaminase (ALT), aspartate aminotransminase (AST), Total protein, albumin, bilirubin, blood urea nitrogen (BUN), creatinine, glucose, uric acid, triglyceride, cholesterol, sodium, potassium and chloride ion. Necropsy was performed to determine gross pathology of various visceral organs. The organs were weighed to determine relative organ weights and then preserved in 10% phosphate buffered formalin. Histological slides of brain, heart, trachea, esophagus, stomach, liver, pancreas, intestines, spleen, bladder, salivary gland, lacrimal gland, thyroid and adrenal glands, reproductive organs (testis and seminal vesicle in male rats, ovary and uterus in female rats) were prepared and histopathologically examined by veterinary pathologist.

Statistical analysis

Body weight, food intake, relative organ weight, hematological and clinical chemistry values were analyzed using one-way analysis (ANOVA) and Bonferroni test was used for multiple comparison at a significant level of $p < 0.05$. Histopathological data were analyzed by Fisher's exact at a significant level of $p < 0.05$.

Results

Acute toxicity

No sign of toxicity and no lethality were observed in either male or female mice receiving suspension of ECa 233 in distilled water in the dose of 10 g/kg within the observation period of 14 days. In addition, no gross pathological lesions were detected in any organs of the experimental animals.

Sub-chronic toxicity

Effects on body weight, food consumption and animal health

Both male and female rats receiving ECa 233 at each dose showed no significant difference in average body weight and food consumption in almost every week when compared with their sex-corresponding control groups except the female treated with the highest dose of ECa 233 had lower food consumption than its respective control exclusively at week 7 (Figures 1 and 2). In addition, no toxic signs or abnormal behavior was found in all ECa 233-treated groups.

Effects on gross pathology and organs weight

At necropsy, no gross pathological lesions were found in control groups of both sexes. In similarity to their sex-corresponding control groups, no observable lesions of the organs were noted in male or female

rats receiving ECa 233. All ECa 233-treated groups showed no significant difference in relative organ weights when compared with their sex-corresponding control groups except male rats receiving ECa 233 at 10 mg/kg/day demonstrated significantly lower relative right adrenal weight than their control group (Tables 1 and 2).

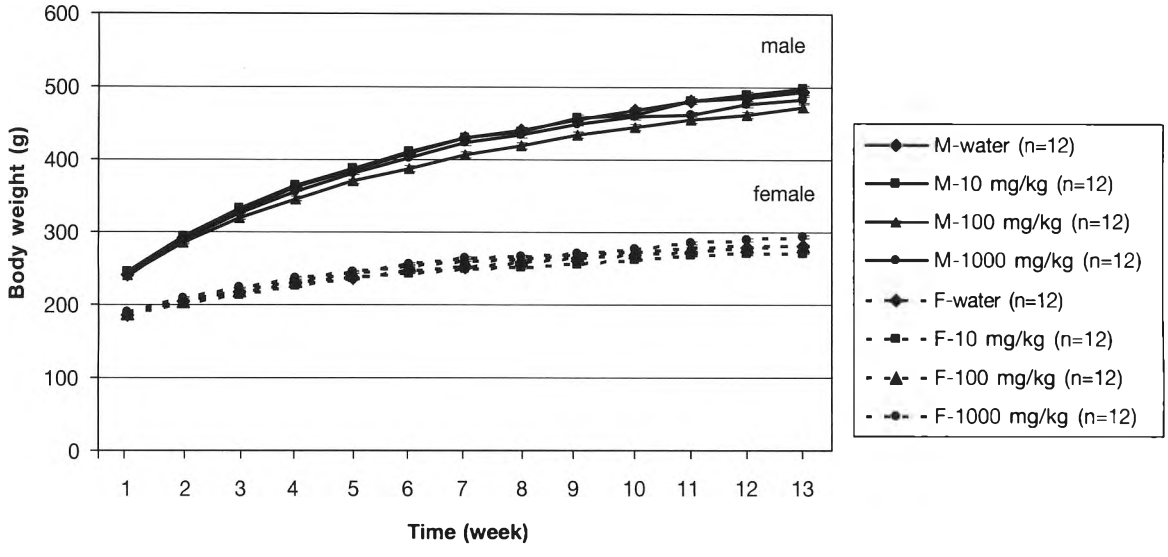

Figure 1 Body growth curves of male (M) and female (F) rats receiving ECa 233 at the doses of 10, 100 and 1000 mg/kg body weight for 3 months

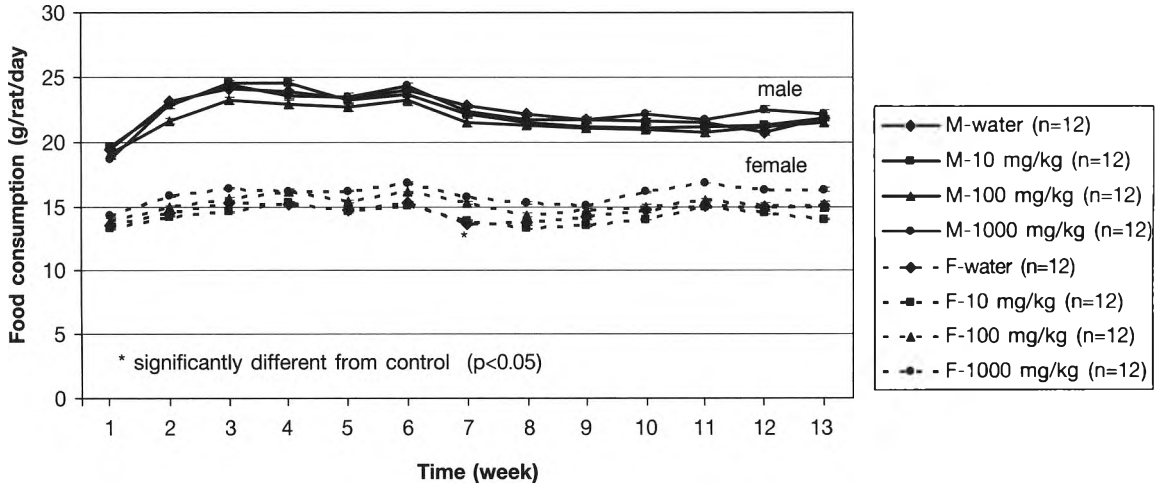

Figure 2 Food consumption of male (M) and female (F) rats receiving ECa 233 at the doses of 10, 100 and 1000 mg/kg body weight for 3 months

Table 1 Relative organ weight (g/1000 g body weight) of male rats receiving ECa 233 for 90 days

Organs	Group of rats receiving ECa 233 (mg/kg/day)			
	0 (n=12)	10 (n=12)	100 (n=11)	1000 (n=12)
Brain	4.48 ± 0.50	4.33 ± 0.38	4.63 ± 0.52	4.57 ± 0.53
Heart	2.69 ± 0.09	2.73 ± 0.22	2.84 ± 0.17	2.79 ± 0.26
Lung	3.15 ± 0.26	3.15 ± 0.27	3.49 ± 0.32	3.34 ± 0.49
Stomach	4.09 ± 0.34	4.06 ± 0.46	4.35 ± 0.41	4.06 ± 0.45
Liver	24.88 ± 1.48	25.46 ± 2.11	25.71 ± 1.33	25.06 ± 1.54
Left kidney	2.35 ± 0.25	2.34 ± 0.23	2.49 ± 0.15	2.49 ± 0.26
Right kidney	2.42 ± 0.17	2.45 ± 0.25	2.57 ± 0.14	2.59 ± 0.24
Spleen	1.73 ± 0.20	1.70 ± 0.17	1.89 ± 0.23	1.69 ± 0.19
Bladder	0.28 ± 0.05	0.31 ± 0.04	0.31 ± 0.06	0.31 ± 0.05
Left testis	6.40 ± 0.73	6.21 ± 0.66	7.12 ± 0.83	6.63 ± 0.66
Right testis	6.29 ± 0.73	5.94 ± 1.05	7.03 ± 0.77	6.87 ± 0.93
Left adrenal gland	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.10 ± 0.02
Right adrenal gland	0.09 ± 0.01	0.07 ± 0.01*	0.09 ± 0.01	0.08 ± 0.02

Data shown were mean ± SD

*Significantly different from control group ($p < 0.05$)

Table 2 Relative organ weight (g/1000 g body weight) of female rats receiving ECa 233 for 90 days

Organs	Group of rats receiving ECa 233 (mg/kg/day)			
	0 (n=12)	10 (n=12)	100 (n=11)	1000 (n=12)
Brain	7.29 ± 0.43	7.49 ± 0.44	7.21 ± 0.36	7.00 ± 0.49
Heart	3.33 ± 0.39	3.38 ± 0.26	3.29 ± 0.28	3.27 ± 0.32
Lung	4.51 ± 0.31	4.62 ± 0.37	4.44 ± 0.54	4.51 ± 0.72
Stomach	5.41 ± 0.44	5.36 ± 0.50	5.24 ± 0.80	5.18 ± 0.65
Liver	25.85 ± 2.75	25.84 ± 2.12	25.71 ± 2.76	25.67 ± 2.49
Left kidney	2.84 ± 0.21	2.96 ± 0.20	2.75 ± 0.20	2.78 ± 0.22
Right kidney	2.89 ± 0.16	3.07 ± 0.22	2.88 ± 0.13	2.91 ± 0.20
Spleen	2.24 ± 0.27	2.19 ± 0.27	2.20 ± 0.34	2.31 ± 0.24
Bladder	0.30 ± 0.03	0.31 ± 0.09	0.27 ± 0.03	0.33 ± 0.05
Left ovary	0.35 ± 0.11	0.35 ± 0.13	0.32 ± 0.10	0.31 ± 0.12
Right ovary	0.33 ± 0.11	0.33 ± 0.09	0.35 ± 0.07	0.31 ± 0.08
Uterus	2.21 ± 0.81	2.20 ± 0.43	2.47 ± 0.76	2.08 ± 0.56
Left adrenal gland	0.17 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	0.17 ± 0.04
Right adrenal gland	0.16 ± 0.04	0.16 ± 0.03	0.16 ± 0.03	0.16 ± 0.04

Data shown were mean ± SD

Effects on hematological parameters

Hematological values in ECa 233-treated male groups were not significantly different from those of their control group (Table 3). Female rats receiving ECa 233 at dose of 1,000 mg/kg/day had a significantly higher number of WBC than their control group. Eosinophils in the female groups receiving ECa 233 at 10 and 1,000 mg/kg/day were significantly lower than those of their control group (Table 4).

Table 3 Hematological values of male rats receiving ECa 233 for 90 days

Parameter	Group of rats receiving ECa 233 (mg/kg/day)			
	0 (n=12)	10 (n=12)	100 (n=11)	1000 (n=12)
Hematocrit (%)	47.36 ± 1.87	46.79 ± 1.91	45.81 ± 1.37	47.03 ± 1.10
Hemoglobin (g/dl)	15.27 ± 0.48	15.08 ± 0.58	14.86 ± 0.41	15.18 ± 0.28
RBC ($x10^6$cells/μL)	8.82 ± 0.24	8.83 ± 0.35	8.54 ± 0.33	8.70 ± 0.26
MCV (fl/red cell)	53.71 ± 1.69	53.04 ± 1.11	53.67 ± 1.34	54.10 ± 1.88
MCH (pg/red cell)	17.32 ± 0.37	17.10 ± 0.33	17.42 ± 0.32	17.46 ± 0.56
MCHC (g/dl RBC)	32.25 ± 0.45	32.25 ± 0.39	32.45 ± 0.46	32.27 ± 0.51
WBC (K/μL)	3.58 ± 1.09	3.80 ± 0.86	3.41 ± 0.94	3.65 ± 0.86
Neutrophil (%)	23.27 ± 5.40	20.94 ± 4.97	26.64 ± 5.14	26.80 ± 4.91
Eosinophil (%)	1.71 ± 0.60	1.43 ± 0.61	1.55 ± 0.49	1.44 ± 0.62
Lymphocyte (%)	71.17 ± 5.76	74.28 ± 5.24	68.38 ± 5.59	68.24 ± 5.87
Monocyte (%)	2.12 ± 2.26	1.88 ± 1.34	2.06 ± 1.36	2.29 ± 1.69
Basophil (%)	1.72 ± 0.65	1.46 ± 0.70	1.37 ± 0.60	1.24 ± 0.67
Platelet (K/μL)	931.50 ± 102.92	957.46 ± 84.17	901.18 ± 68.34	915.00 ± 64.61

Data shown were mean ± SD

Table 4 Hematological values of female rats receiving ECa 233 for 90 days

Parameter	Group of rats receiving ECa 233 (mg/kg/day)			
	0 (n=12)	10 (n=12)	100 (n=12)	1000 (n=12)
Hematocrit (%)	46.01 ± 1.66	46.15 ± 1.80	45.56 ± 1.23	46.63 ± 2.42
Hemoglobin (g/dl)	14.91 ± 0.54	14.92 ± 0.66	14.90 ± 0.40	15.10 ± 0.77
RBC ($x10^6$cells/μL)	8.06 ± 0.42	8.10 ± 0.39	7.96 ± 0.24	8.20 ± 0.56
MCV (fl/red cell)	57.15 ± 1.92	56.98 ± 1.74	57.28 ± 1.13	56.95 ± 1.21
MCH (pg/red cell)	18.51 ± 0.75	18.42 ± 0.63	18.71 ± 0.29	18.45 ± 0.43
MCHC (g/dl RBC)	32.39 ± 0.36	32.33 ± 0.98	32.67 ± 0.40	32.38 ± 0.29
WBC (K/μL)	1.90 ± 0.57	2.28 ± 0.52	2.26 ± 0.57	2.77 ± 1.09*
Neutrophil (%)	21.85 ± 4.91	18.80 ± 6.26	21.35 ± 5.37	17.26 ± 6.54
Eosinophil (%)	2.02 ± 0.68	1.38 ± 0.58*	1.55 ± 0.42	1.29 ± 0.48*
Lymphocyte (%)	73.35 ± 5.86	76.56 ± 7.59	74.91 ± 5.42	78.89 ± 6.91
Monocyte (%)	1.76 ± 1.73	2.05 ± 2.26	1.38 ± 0.74	1.55 ± 0.94
Basophil (%)	1.03 ± 0.54	1.22 ± 0.59	0.81 ± 0.33	1.03 ± 0.34
Platelet (K/μL)	965.67 ± 84.28	928.42 ± 166.88	918.25 ± 94.56	912.70 ± 84.02

Data shown were mean ± SD

*Significantly different from control group ($p < 0.05$)

Effects on clinical chemistry parameters

Clinical chemistry values of all ECa 233-treated, male and female, groups were not significantly different from those of their sex-corresponding control groups except male rats receiving the highest dose had slightly but significantly higher sodium level than their control groups (Tables 5 and 6).

Histopathology

Male rats receiving ECa 233 at 10 mg/kg/day had a significantly higher incidence of lymphoid proliferation in the lung and centrilobular fatty degeneration in the liver than their control group (Table 7). However, there was clearly no exacerbation of changes or increase of effect at higher dose of 100 and 1,000 mg/kg/day were noted. The incidences of GALT proliferation in the small intestine of female rats receiving ECa 233 at the doses of 10 and 1,000 mg/kg/day were significantly lower than those observed in their respective control groups (Table 8).

Discussion

The oral acute toxicity study revealed that ECa 233 at a single dose of 10 g/kg which was the highest dose that could be given to mice produced no acute toxic effect, lethality and gross pathological lesions. Therefore, LD_{50} value in mice should be more than 10 g/kg suggesting a relatively high margin of safety of ECa 233 in relation to its effective dose in enhancing memory at 10 and 30 mg/kg [6]. Considering that *C. asiatica* is a common edible plant and, in previous study, oral dose of dried *C. asiatica* at 8 g/kg has been shown to cause no sign of acute toxicity [4]. This finding is not surprising.

Subchronic toxicity in Wistar rats orally given with ECa 233 at doses of 10, 100 and 1,000 mg/kg/day which were equivalent to 1, 10 and 100 fold of effective dose in improving learning and memory in rodents [6] revealed that ECa 233 did not affect body weight, food consumption and animal health. The decrease of the right adrenal relative weight in male rats receiving ECa 233 at 10 mg/kg/day seems unlikely to be accounted by ECa 233 as such effect was not detected in animals receiving 100 or 1,000 mg/kg. The significant increase of WBC in female rats receiving the highest dose of ECa 233 agrees well with the finding that methanol

Table 5 Clinical chemistry value of male rats receiving ECa 233 for 90 days

Parameter	Group of rats receiving ECa 233 (mg/kg/day)			
	0 (n=12)	10 (n=12)	100 (n=12)	1000 (n=12)
ALT (U/L)	33.58 ± 9.58	30.25 ± 6.97	30.00 ± 4.61	27.92 ± 2.97
AST (U/L)	96.75 ± 13.46	94.25 ± 16.21	97.25 ± 17.09	100.17 ± 15.64
ALP (U/L)	62.00 ± 7.93	61.00 ± 8.29	60.75 ± 8.00	62.08 ± 7.28
BUN (mg/dl)	18.49 ± 2.68	20.48 ± 2.57	19.38 ± 1.99	19.73 ± 3.13
Creatinine (mg/dl)	0.54 ± 0.06	0.56 ± 0.10	0.54 ± 0.06	0.61 ± 0.12
Total protein (g/dl)	6.51 ± 0.31	6.51 ± 0.19	6.48 ± 0.29	6.42 ± 0.33
Albumin (g/dl)	4.41 ± 0.14	4.42 ± 0.17	4.49 ± 0.13	4.49 ± 0.13
Bilirbubin (mg/dl)	0.08 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.08 ± 0.03
Glucose (mg/dl)	184.19 ± 24.28	181.00 ± 29.59	177.07 ± 20.52	176.79 ± 25.57
Uric acid (mg/dl)	1.99 ± 0.77	1.91 ± 1.08	1.79 ± 0.98	1.77 ± 0.80
Triglyceride (mg/dl)	108.09 ± 46.32	106.64 ± 46.69	70.52 ± 39.42	79.78 ± 33.49
Cholesterol (mg/dl)	59.51 ± 11.01	63.17 ± 11.89	53.82 ± 7.37	65.76 ± 10.50
Sodium (mmol/l)	145.83 ± 0.94	145.83 ± 0.94	147.00 ± 1.28	147.75 ± 1.22*
Potassium (mmol/l)	5.07 ± 0.82	4.88 ± 0.99	4.87 ± 0.86	4.20 ± 0.54
Chloride (mmol/l)	106.83 ± 1.19	106.42 ± 0.90	107.50 ± 1.73	107.83 ± 1.27

Data shown were mean ± SD

*Significantly different from control group ($p < 0.05$)

extract of *C. asiatica* has been shown to increase total WBC count in mice [9]. Besides, the water extract of *C. asiatica* was demonstrated to significantly increase pokeweed mitogen-induced lymphocyte proliferation *in vitro* [10]. However, the increase of WBC in the highest dose-treated group could not be classified as leucocytosis because the WBC in the control group itself was lower than rat normal range [11,12]. The decrease of eosinophil in female rats receiving 10 and 1,000 mg/kg/day was within normal range [11] and showed no dose dependency and thus might not be related to ECa 233. Dose-related increase in the level of sodium was observed, however, it was within normal range [12]. The significant increases of bronchiole associated lymphoid tissue proliferation in the lung and centrilobular fatty degeneration in the liver were observed exclusively in the male rat group receiving 10 mg/kg of ECa 233. Inconsistency and lack of dose-dependency in the finding observed do not support causal effect relationship between ECa 233 and the lesions. In contrast to liver specific toxicity of dried aerial part of *C. asiatica* previously reported [8], ECa 233 at the dose of 1,000 mg/kg showed no sign of hepatotoxic in terms of liver enzymes or histopathology. Differences in composition of the test compounds, crude powder in previous study versus standardized extract in the present study, could possibly account for the discrepancy observed. Moreover, the finding that small intestinal associated lymphoid tissue proliferation in the female rats receiving ECa 233 at the doses of 10 and 1,000 mg/kg was significantly lower than that of control might suggest its protective effect. Moreover, no other histopathological observations in the ECa 233-treated groups were found to be significantly different from those of their respective control groups. Taken all together the present study has demonstrated a good safety profile of a standardized extract of *C. asiatica* in both acute and sub-chronic toxicity testing. Based on the fact that ECa 233 is derived from edible plant with proof of benefit and safety on top of its favorable physical property with regards to consistency of active ingredients and being colorless, it is noteworthy to further develop health food or pharmaceutical products from the standardized extract of *C. asiatica*.

Table 6 Clinical chemistry values of female rats receiving ECa 233 for 90 days

Parameter	Group of rats receiving ECa 233 (mg/kg/day)			
	0 (n=12)	10 (n=12)	100 (n=12)	1000 (n=12)
ALT (U/L)	25.17 ± 3.38	23.58 ± 4.72	23.75 ± 2.38	21.83 ± 4.43
AST (U/L)	89.00 ± 10.25	83.42 ± 8.37	86.58 ± 6.40	86.33 ± 17.03
ALP (U/L)	31.00 ± 8.25	29.58 ± 10.41	30.42 ± 7.66	29.00 ± 11.34
BUN (mg/dl)	21.98 ± 4.56	23.71 ± 6.25	23.13 ± 4.48	22.05 ± 4.20
Creatinine (mg/dl)	0.62 ± 0.13	0.61 ± 0.14	0.66 ± 0.10	0.61 ± 0.14
Total protein (g/dl)	6.57 ± 0.23	6.48 ± 0.41	6.65 ± 0.27	6.58 ± 0.48
Albumin (g/dl)	4.67 ± 0.16	4.65 ± 0.20	4.85 ± 0.17	4.76 ± 0.39
Bilirbubin (mg/dl)	0.09 ± 0.04	0.11 ± 0.03	0.11 ± 0.04	0.11 ± 0.03
Glucose (mg/dl)	138.39 ± 15.43	137.44 ± 19.12	130.47 ± 14.65	140.17 ± 22.19
Uric acid (mg/dl)	1.38 ± 0.53	1.53 ± 0.70	1.39 ± 0.57	1.24 ± 0.43
Triglyceride (mg/dl)	37.17 ± 8.33	35.70 ± 8.54	39.68 ± 8.38	37.47 ± 4.74
Cholesterol (mg/dl)	52.6 ± 12.58	49.32 ± 11.21	52.81 ± 12.16	54.73 ± 10.43
Sodium (mmol/l)	146.17 ± 1.53	146.17 ± 1.11	146.83 ± 1.03	146.83 ± 1.40
Potassium (mmol/l)	4.47 ± 0.55	4.48 ± 0.72	4.17 ± 0.80	4.19 ± 0.83
Chloride (mmol/l)	109.75 ± 1.22	109.42 ± 2.15	109.75 ± 1.22	108.92 ± 1.24

Data shown were mean < SD

Table 7 Histopathological results of male rats receiving ECa 233 for 90 days

Organs	Histopathological alteration	Group of rats receiving ECa 233 (mg/kg/day)			
		0	10	100	1000
Lung	Bronchiolar associated lymphoid tissue proliferation	1/12	6/12*	3/12	5/12
Heart	Focal myocardiosis	3/12	1/12	0/12	1/12
Liver	Centrilobular fatty degeneration	0/12	5/12*	3/12	1/12
	Small necrosis with mononuclear portal area	1/12	1/12	1/12	0/12
Kidney	Hydronephrosis	0/12	1/12	0/12	0/12
Small intestine	Gut associated lymphoid tissue proliferation	2/12	1/12	1/12	1/12
Large intestine	Gut associated lymphoid tissue proliferation	1/12	2/12	2/12	3/12
Adrenal gland	Cortical fatty infiltration	0/12	2/12	2/12	0/12

Data shown were number of rat with histopathogical alteration/total number of rat in each group

*Significantly different from control group ($p < 0.05$)

Table 8 Histopathological results of female rats receiving ECa 233 for 90 days

Organs	Histopathological alteration	Group of rats receiving ECa 233 (mg/kg/day)			
		0	10	100	1000
Lung	Bronchiole associated tissue proliferation	2/12	4/12	3/12	5/12
Liver	Multifocal necrosis at portal	0/12	3/12	1/12	0/12
Kidney	Hydronephrosis	1/12	1/12	0/12	1/12
Small intestine	Gut associated lymphoid proliferation	5/12	0/12*	3/12	0/12*
Large intestine	Gut associated lymphoid proliferation	1/12	2/12	2/12	2/12

Data shown were number or rats with alteration/total number or rats in each group

*Significantly different from control group ($p < 0.05$)

Conclusion

Acute toxicity study of the standardized extract of *Centella asiatica* ECa 233 in mice by oral administration a single dose of 10 g/kg which was equivalent to 1,000 times of effective dose in enhancing memory effect in rodent did not produce any toxic signs, mortality and gross pathological lesions. Subchronic toxicity study of ECa 233 in Wistar rats by oral administration at the doses of 10, 100 and 1,000 mg/kg/day showed that the extract did not affect body weight, food consumption and animals health Female rats receiving ECa 233 at 1,000 mg/kg/day had a significant increase in WBC; but could not be regarded as leucocytosis. Clinical chemistry values in ECa 233-treated rats were not significantly different from those of their corresponding sex-control groups except for a significant increase of sodium level in the male rats receiving the highest dose. However, this change was still within normal ranges. ECa 233 did not cause any significant microscopic changes of vital organs. Histopathological results revealed no causal

effect relationship between ECa 233 and the lesions. Taken all together, ECa 233 is safe and should be further developed to pharmaceutical products or food supplement.

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