Original Article

Acute and Subchronic Toxicity Study of *Ardisia ellipica* Thunb. Fruit Extract

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Abstract

Several plants species in the genus *Ardisia* contained various biologically active compounds. Ram Yai or Pilangkasa (*Ardisia elliptica* Thunb.) has been used in traditional medicines. The objective of this study was to evaluate the safety of ethanolic extract of *A. elliptica* fruit in animal models by oral administration. Acute toxicity test in mice by gavage the extract at each dose of 2.5 g/kg twice revealed no abnormal signs, mortality and gross lesion of vital organs. Subchronic toxicity study was investigated in one hundred Wistar rats separated into five groups, each of twenty (ten males and ten females). Two control groups received distilled water and 0.5% tragacanth respectively. While three experimental groups were orally administered with the extract at the doses of 20, 200 and 2000 mg/kg/day for 90 days consecutively. The results revealed that the extract at different doses did not affect growth, food consumption, health status, organ weights and clinical chemistry values of the rats. Hematological results revealed that the male rats receiving the extract at 200 mg/kg had higher MCHC value than the water control group; however it did not show any dose dependency. The significant alterations of neutrophil and eosinophil in the highest dose-treated male group were within the reference range. The incidence of histopathological lesions in some organs did not show any dose response relationship. In conclusion, the ethanolic extract of Ram Yai fruit at the tested doses did not produce any acute and subchronic toxicity in experimental animals.

Keywords: *A. elliptica* extract, acute, mice, rat, subchronic toxicity

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บทคัดย่อ

พิษเฉียบพลันและพิษกึ่งเรื้อรังของสารสกัดรามใหญ่

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พืชในสกุล *Ardisia* หลายชนิดมีองค์ประกอบทางเคมีที่มีฤทธิ์ทางชีวภาพที่น่าสนใจหลายประการ รามใหญ่หรือพิลังกาสาเป็นสมุนไพรชนิดหนึ่งในสกุลนี้ที่มีการใช้ในตำราแพทย์แผนโบราณ การศึกษาครั้งนี้มีวัตถุประสงค์ เพื่อประเมินความปลอดภัยของสารสกัดจากผลรามใหญ่ในสัตว์ทดลอง โดยวิธีป้อนทางปาก จากการทดสอบพิษเฉียบพลันในหนูถีบจักรโดยกรอกสารสกัดสองครั้งๆละ 2.5 ก./กก. พบว่าไม่ก่อให้เกิดอาการผิดปกติใดๆและไม่ทำให้หนูเสียชีวิต ผลการศึกษาพิษกึ่งเรื้อรังในหนูแรทพันธุ์วิสตาร์ จำนวน 100 ตัว แบ่งออกเป็น 5 กลุ่มๆ ละ 20 ตัว (เพศละ 15 ตัว) โดยกลุ่มควบคุมสองกลุ่มได้รับน้ำกลั่นและสารละลายทรากาคานต์ ส่วนกลุ่มทดลอง 3 กลุ่มได้รับสารสกัดรามใหญ่ขนาด 20, 200 และ 2000 มก./กก./วัน ติดต่อกันทุกวันเป็นเวลา 90 วัน พบว่า สารสกัดรามใหญ่ไม่มีผลต่อน้ำหนักตัว สุขภาพ น้ำหนักอวัยวะสัมพัทธ์ และค่าทางเคมีคลินิกแต่อย่างใด การตรวจค่าทางโลหิตวิทยาพบว่า หนูเพศผู้กลุ่มที่ได้รับสารสกัดขนาด 200 มก./กก./วัน มีค่า MCHC สูงกว่ากลุ่มควบคุมด้วยน้ำอย่างมีนัยสำคัญแต่ไม่สัมพันธ์กับขนาดที่ได้รับ และกลุ่มที่ได้รับสารสกัดขนาด 2000 มก./กก./วัน มีเซลล์นิวโทรฟิลลดลง แต่เซลล์อิโอสิโนฟิล เพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับกลุ่มควบคุมด้วยน้ำแต่ยังคงอยู่ในช่วงค่าปกติของหนูแรท ผลทางพยาธิวิทยา พบรอยโรคทางจุลพยาธิวิทยาได้ในบางอวัยวะอย่างไม่สัมพันธ์กับขนาดของสารสกัดรามใหญ่ ดังนั้นสรุปได้ว่า สารสกัดรามใหญ่หรือพิลังกาสาไม่ก่อให้เกิดพิษเฉียบพลันและพิษกึ่งเรื้อรังต่อสัตว์ทดลอง

คำสำคัญ: สารสกัดรามใหญ่ พิษเฉียบพลัน พิษเรื้อรัง หนูไมซ์ หนูแรท

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Introduction

The genus *Ardisia* is the largest in the family Myrsinaceae, and several species produce several groups of biologically active phytochemicals such as peptides, saponins, isocoumarins quinines and alkylphenols (Kobayashi and Mejia, 2005). Of these, *Ardisia elliptica* Thunb., also known in Thailand as Ram Yai or Pilangkasa, is one of the interesting medicinal plants. In traditional medicines, the Malaysians used the decoction of the leaves to treat chest pain, herpes and measles. The fruits were prescribed in Thai traditional medicines for curing diarrhea with fever (Ling et al., 2009). The crude extract and some phytochemical constituents from *A. elliptica* were proven to possess various biological activities. Ethanolic extract of the fruit showed antiproliferative activity on human breast adenoma cell line (Moongkarndi et al., 2004). β- amyrin exhibited more potent activity than aspirin in inhibiting platelet aggregation (Ching et al., 2010). Berginine exhibiting a wide range of biological activities including hepatoprotective, antifungal, antiarrhythmic and hypolipidemic was found in the highest content in *A. elliptica* when compared with other 16 species naturally occurring in China (Kobayashi and Mejia, 2005). Embellin was demonstrated to possess analgesic (Atal et al., 1984), wound healing (Swamy et al., 2007) antibacterial (Chitra et al., 2003) and antioxidant (Joshi et al., 2007). Recently, embellin extracted from the fruits of *A. elliptica* showed high potency of antiglycation activity and it has been developed for external use as cosmeticeutics for aging (Shuayprom et al., 2010). Even though phytochemicals of *A. elliptica* indicate that it has potential for health products development, toxicological data of this plant has not been fully explored. The objectives of this study were to evaluate the safety of the ethanolic extract of *A. elliptica* fruits in animal models and the results may be useful to support the utilization of health products from *A. elliptica*.

Materials and Methods

Ardisia elliptic extract (AE): The fruits of *A. elliptica* were collected from the plantations in The Medicinal Plant Garden of the Department of Medical Sciences, Rayong Province, Thailand**.** The plant specimens were identified by Rachan Pooma, Office of the Forest Herbarium, Department of National Parks, Wildlife

and Plant Conservation. A voucher specimen of *A. elliptica* (BKF No. 110703) was deposited at the Forest Herbarium, Thailand. The dried fruits of *A. elliptica* were coarsely pulverized into powder. The powder was extracted with 95% ethanol using soxhlet apparatus. The extract solution was evaporated using rotary evaporator at 50^0C under reduced pressure and then the concentrated extract was dried using lyophilizer. The yield of dried extract from the dried fruits was about 28.28% (w/w). Embellin, a major biological active compound in AE, was found to be 2.04% according to HPTLC analysis. The extract was kept in well-closed container, protected from light at -20^0C for further toxicological investigation.

Animals: Thirty ICR mice (15 males and 15 females weighing 20-22 g) and one hundred Wistar rats (50 males weighing 180-200 g and 50 females weighing 170-190 g) were purchased from The National Laboratory Animal Center, Mahidol University. The animals were housed in a hygienic conventional animal room of the Laboratory Animal Center, Department of Medical Sciences where the environment of the room was maintained at 25±1^0C with 60% humidity and 12 hour-light-dark cycle. They were raised with commercial pellet diet (082 CP® feed, Perfect Companion Group, Thailand) and clean water *ad libitum*. The mice were fasted for two hours before acute toxicity test. Prior to the subchronic toxicity study, the rats were acclimatized with the environment for two weeks. The protocol study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Department of Medical Sciences (Code No. 53-011).

Acute toxicity test: The mice were randomly divided into three groups, each of ten animals (five males and five females). The experimental group was orally given AE suspension at dose of 2.5 g/kg while two control groups received distilled water and tragacanth solution at the volume of 10 ml/kg respectively. The animals were observed for four hours and the process was then repeated. Following administration, they were observed for abnormal signs and mortality for 14 days. At the end of the observation period, the mice were sacrificed with CO_2 inhalation and necropsy was performed to examine gross pathology of their visceral organs.

Subchronic toxicity study: The Wistar rats were randomly divided into five groups of ten animals of each sex. Group1 and 2 were control groups receiving distilled water and 0.5% tragacanth at the volume of 10 ml/kg, respectively. Group 3 to 5 were experimental groups orally administered with AE suspension at the doses of 20, 200 and 2000 mg/kg/day for 90 days consecutively. During the experimental period, body weight and food intake were recorded weekly and the animals were observed for general appearance, behavior and signs of abnormalities. At the end of the 90-day treatment period, the animals were fasted overnight, anesthesized with diethyl ether inhalation. Blood samples were collected from posterior vena cava for determining hematological and serum clinical chemistry values.

Hematological analysis was performed using automatic hematological analyzer Cell Dyn® 3500 (Abbot Laboratories Ltd, USA). Parameters examined were hematocrit (Hct), hemoglobin, erythrocyte (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell (WBC), neutrophil, eosinophil, lymphocyte, monocyte, basophil and platelet.

Clinical chemistry values were measured by using automatic chemistry analyzer Cobas®Integra 400 plus (Roche Diagnostics Ltd, Switzerland) and parameters assayed were alkaline phosphatase (ALP), alanine transminase (ALT), aspartate transminase (AST), total protein, albumin, bilirubin, blood urea nitrogen (BUN), creatinine, glucose, uric acid, triglyceride, cholesterol, sodium, potassium and chloride ions.

A complete necropsy was performed to determine gross lesions of various visceral organs. Brain, heart, lung, liver, kidney, stomach, spleen, testis, uterus, urinary bladder and adrenal glands were weighed by using Mettler Toledo® PB 153 balance (Metler Toledo International Inc, Switzerland). Organ weights were calculated into relative organ weight (g/1000 g body weight). The visceral organs were fixed in 10% buffered formalin, and subjected to conventional histological process. Histopathological examination was performed on the above mentioned organs including trachea, lymph node, esophagus, pancreas, intestines, thyroid gland, lacrimal and salivary glands, prostate gland, seminal vesicle, ovary, uterus, and mammary glands.

Statistical analysis: The data were statistically evaluated by one way ANOVA. Comparison between treatment and control groups were made by Bonferroni test. For histopathological results, Fisher's exact was applied. Differences between groups were considered significant at $p<0.05$.

Results

Acute toxicity test: The mice receiving AE at the dose of 2.5 g/kg twice did not manifest any abnormal signs or behaviors during the observation period. All AE-treated mice survived until the end of the experiment. Necropsy revealed no gross lesions in the visceral organs when compared with both control groups.

Subchronic toxicity study

***Effect of AE on body weight, relative organ weights, food consumption and health status*:** Both male and female rats receiving AE at the doses of 20, 200 and 2000 mg/kg/day showed no significant difference in their average body weight (Fig 1) and relative organs weight (Table 1 and 2) when compared with their corresponding water and tragacanth control groups. Measurement of the weekly food intake in the AE-treated male rats showed no significant difference over the whole experimental period as compared with the corresponding control groups. In the female, only the highest dose-treated group had significantly lower food intake than the tragacanth control group at week 6, 7, 9 and than the water control group at only

week10 (Fig 2). All of the AE-treated groups revealed healthy and showed no sign of abnormality, as compared to both control groups.

Effects of ME on hematological values: Male rats receiving AE at dose of 200 mg/kg/day had significantly higher MCHC than the water control group. The highest dose-treated male rats had significantly higher neutrophil but significantly lower eosinophil when compared with those of the water control group (Table 3). In the female, there was no significant difference in any hematological parameters between the treatment and control groups (Table 4).

Effects of ME on clinical chemistry values: Both male and female rats receiving AE had no significant difference in any of their clinical chemistry values as compared with the corresponding control groups (Table 5 and 6).

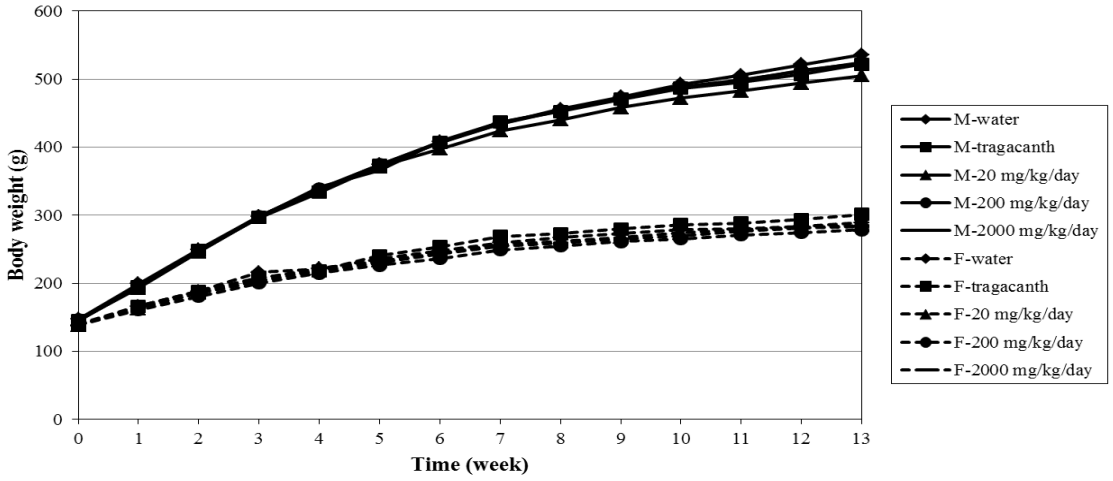

Figure 1 Growth curves of male (M) and female (F) rats receiving AE for 90 days.

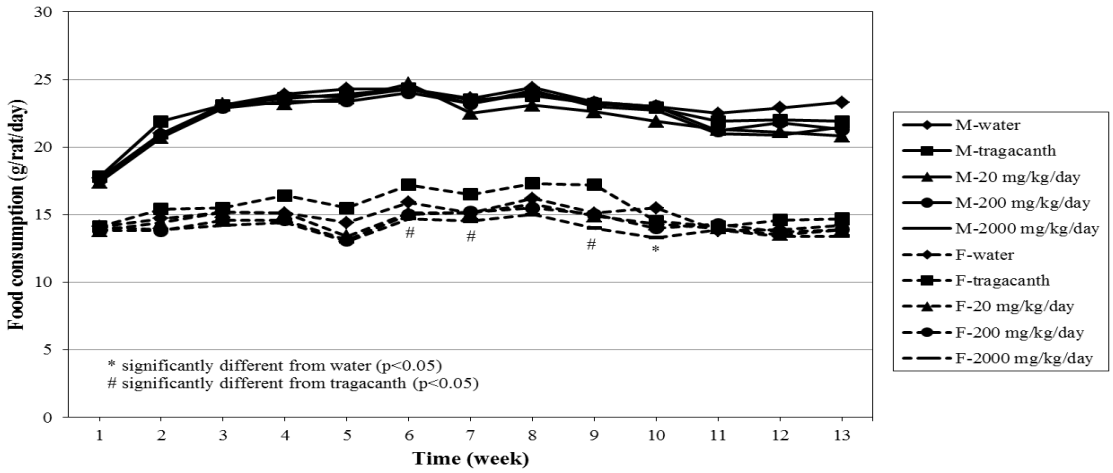

Figure 2 Food consumption of male (M) and female (F) rats receiving AE for 90 days.

Table 1 Relative organ weight (g/1000g of body weight) of male rats receiving AE for 90 days
The values are expressed as mean±SD, DW: Distilled water, TG: Tragacanth

Organs	Dose of AE (mg/kg/day)				
	DW	0.5% Tragacanth	20	200	2000
	n = 10	n = 10	n = 10	n = 10	n = 10
Brain	4.08 ± 0.30	4.16 ± 0.45	4.27 ± 0.31	4.16 ± 0.24	4.09 ± 0.38
Heart	2.88 ± 0.13	2.83 ± 0.12	2.81 ± 0.14	2.74 ± 0.13	2.82 ± 0.15
Lung	3.18 ± 0.21	3.16 ± 0.34	3.16 ± 0.16	3.14 ± 0.21	3.02 ± 0.29
Liver	27.40 ± 2.55	28.24 ± 2.01	27.54 ± 2.24	27.50 ± 2.01	28.51 ± 2.34
Stomach	4.10 ± 0.38	4.08 ± 0.45	4.00 ± 0.47	4.11 ± 0.43	4.23 ± 0.24
Spleen	1.74 ± 0.15	1.74 ± 0.19	1.77 ± 0.22	1.66 ± 0.17	1.73 ± 0.18
Right kidney	2.52 ± 0.20	2.61 ± 0.14	2.50 ± 0.16	2.49 ± 0.21	2.53 ± 0.18
Left kidney	2.43 ±0.19	2.43 ± 0.15	2.44 ± 0.16	2.36 ± 0.17	2.45 ± 0.20
Right testis	5.70 ± 0.62	5.82 ± 0.54	5.76 ± 0.58	5.62 ± 0.49	5.65 ± 0.53
Left testis	5.77 ± 0.71	5.87 ± 0.54	5.80 ± 0.49	5.50 ± 0.42	5.68 ± 0.47
Right adrenal	0.08 ±0.01	0.07 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Left adrenal	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.02
Bladder	0.28 ± 0.05	0.31 ± 0.05	0.28 ± 0.07	0.27 ± 0.04	0.29 ± 0.06

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

Organs	Dose of AE (mg/kg/day)				
	DW	0.5% TG	20	200	2000
	n = 10	n = 10	n = 10	n = 10	n = 10
Brain	6.91 ± 0.81	6.84 ± 0.55	7.09 ± 0.38	7.31 ± 0.42	7.08 ± 0.51
Heart	3.34 ± 0.34	3.23 ± 0.25	3.32 ± 0.24	3.31 ± 0.14	3.22 ± 0.21
Lung	4.30 ± 0.31	4.23 ± 0.25	4.32 ± 0.31	4.31 ± 0.26	4.23 ± 0.48
Liver	27.25 ± 2.22	26.51 ± 2.63	27.25 ± 2.91	27.23 ± 2.15	27.47 ± 2.86
Stomach	5.62 ± 0.52	4.85 ± 1.05	5.48 ± 0.66	5.30 ± 0.37	5.28 ± 0.66
Spleen	2.25 ± 0.22	2.18 ± 0.16	2.21 ± 0.21	2.18 ± 0.16	2.36 ± 0.43
Right kidney	2.92 ± 0.19	2.88 ± 0.12	2.86 ± 0.19	3.04 ± 0.28	2.95 ± 0.29
Left kidney	2.75 ± 0.15	2.66 ± 0.24	2.68 ± 0.15	2.90 ± 0.25	2.79 ± 0.33
Right adrenal	0.15 ± 0.04	0.15 ± 0.02	0.16 ± 0.04	0.17 ± 0.02	0.15 ± 0.03
Left adrenal	0.17 ± 0.04	0.16 ± 0.01	0.18 ± 0.03	0.17 ± 0.02	0.16 ± 0.03
Bladder	0.33 ± 0.04	0.27 ± 0.03*	0.32 ± 0.09	0.29 ± 0.04	0.31 ± 0.03
Uterus	2.24 ± 0.66	1.94 ± 0.46	2.03 ± 0.73	1.97 ± 0.27	2.08 ± 0.50
Right ovary	0.27 ± 0.06	0.28 ± 0.05	0.28 ± 0.04	0.30 ± 0.04	0.30 ± 0.06
Left ovary	0.33 ± 0.06	0.32 ± 0.08	0.31 ± 0.07	0.33 ± 0.05	0.30 ± 0.09

The values are expressed as mean±SD
*significantly different from water control group (p< 0.05), DW: Distilled water, TG: Tragacanth

Table 3 Hematological values of male rats receiving AE for 90 days

Parameters	Dose of AE (mg/kg/day)				
	DW	0.5% TG	20	200	2000
	n = 10	n = 10	n = 10	n = 10	n = 10
Hematocrit (%)	34.36 ± 1.44	34.08 ± 1.52	34.47 ± 1.54	33.17 ± 1.06	32.75 ± 0.73
Hemoglobin (g/dl)	16.20 ± 0.75	16.16 ± 0.65	16.29 ± 0.67	15.87 ± 0.49	15.63 ± 0.42
RBC (x 10^6 cells/mm^3)	9.25 ± 0.50	9.36 ± 0.39	9.33 ± 0.41	9.13 ± 0.37	8.87 ± 0.28
MCV (fl/red cell)	37.14 ± 1.00	36.43 ± 0.82	36.97 ± 0.88	36.35 ± 0.65	36.96 ± 0.77
MCH (pg/red cell)	17.53 ± 0.44	17.29 ± 0.40	17.48 ± 0.40	17.40 ± 0.42	17.64 ± 0.39
MCHC (g/dl RBC)	47.17 ± 0.41	47.44 ± 0.40	47.30 ± 0.43	47.85 ± 0.64*	47.70 ± .45
WBC (x 10^3 cells/mm^3)	3.35 ± 0.62	3.56 ± 0.59	3.55 ± 0.79	3.33 ± 0.56	3.29 ± 1.23
Neutrophil (%)	21.64 ± 3.13	24.56 ± 6.17	21.58 ± 2.83	24.71 ± 5.57	28.65 ± 4.82*
Eosinophil (%)	1.91 ± 0.56	1.53 ± 0.69	1.72 ± 0.49	2.01 ± 0.75	1.00 ± 0.21*
Lymphocyte (%)	74.35 ± 3.12	71.07 ± 7.20	75.38 ± 2.82	71.28 ± 5.68	68.20 ± 5.41
Monocyte (%)	1.40 ± 1.49	2.20 ± 3.02	0.86 ± .25	1.34 ± 1.36	1.43 ± 0.88
Basophil (%)	0.72 ± 0.45	0.65 ± 0.61	0.45 ± 0.21	0.68 ± 0.38	0.73 ± 0.31
Platelet (x10^3 cells/mm^3)	955.35 ± 98.01	1,028.10 ± 85.29	956.00 ± 76.20	1,020.20 ± 108.23	968.00 ± 116.54

The values are expressed as mean±SD
*significantly different from water control group (p< 0.05), DW: Distilled water, TG: Tragacanth

Table 4 Hematological values of female rats receiving AE for 90 days

Parameters	Dose of AE (mg/kg/day)				
	DW	0.5% TG	20	200	2000
	n = 10	n = 10	n = 10	n = 10	n = 10
Hematocrit (%)	31.70 ± 5.07	33.02 ± 1.70	33.17 ± 1.35	32.55 ± 1.77	32.53 ± 1.52
Hemoglobin (g/dl)	16.06 ± 0.63	15.77 ± 0.83	15.93 ± 0.66	15.59 ± 0.88	15.80 ± 0.65
RBC (x 10^6 cells/mm^3)	8.54 ± 0.42	8.48 ± 0.57	8.48 ± 0.40	8.33 ± 0.48	8.43 ± 0.42
MCV (fl/red cell)	39.20 ± 0.74	38.98 ± 1.16	39.13 ± 0.68	39.07 ± 0.73	38.58 ± 0.89
MCH (pg/red cell)	18.83 ± 0.41	18.61 ± 0.52	18.81 ± 0.45	18.71 ± 0.34	18.78 ± 0.77
MCHC (g/dl RBC)	48.00 ± 0.41	47.76 ± 0.55	48.02 ± 0.53	47.88 ± 0.58	48.69 ± 1.75
WBC (x 10^3 cells/mm^3)	1.83 ± 0.50	2.06 ± 0.67	1.75 ± 0.49	1.76 ± 0.49	1.93 ± 0.56
Neutrophil (%)	24.55 ± 5.73	26.56 ± 8.19	27.12 ± 4.69	24.26 ± 3.89	25.33 ± 8.48
Eosinophil (%)	1.86 ± 1.00	1.68 ± 0.86	2.05 ± 1.17	1.77 ± 0.94	1.36 ± 0.56
Lymphocyte (%)	71.76 ± 6.00	68.93 ± 8.06	69.25 ± 4.31	72.21 ± 3.73	70.43 ± 8.33
Monocyte (%)	1.17 ± 0.59	1.98 ± 1.84	1.04 ± 0.44	1.22 ± 0.40	2.07 ± 1.30
Basophil (%)	0.66 ± 0.33	0.82 ± 0.50	0.54 ± 0.21	0.52 ± 0.29	0.81 ± 0.30
Platelet (x10^3 cells/mm^3)	896.80 ± 114.91	895.45 ± 55.13	891.30 ± 101.59	906.85 ± 88.51	918.05 ± 56.66

The values are expressed as mean±SD, DW: Distilled water, TG: Tragacanth

Table 5 Biochemical values of male rats receiving AE for 90 days

Parameters	Dose of AE (mg/kg/day)				
	DW	0.5% TG	20	200	2000
	n = 10	n = 10	n = 10	n = 10	n = 10
ALP(U/l)	54.00 ± 8.93	51.80 ± 6.53	52.50 ± 4.86	52.40 ± 5.99	53.00 ± 5.29
ALT(U/l)	30.20 ± 5.01	29.10 ± 6.08	27.90 ± 3.48	29.40 ± 5.74	29.00 ± 6.85
AST(U/l)	89.00 ± 8.92	82.80 ± 6.88	84.00 ± 9.63	83.90 ± 10.08	79.30 ± 9.15
BUN (mg/dl)	20.45 ± 2.92	20.21 ± 3.61	18.69 ± 2.30	20.35 ± 3.19	18.46 ± 3.50
Creatinine (mg/dl)	0.54 ± 0.06	0.53 ± 0.11	0.49 ± 0.04	0.48 ± 0.06	0.48 ± 0.08
Total Protein (g/dl)	6.38 ± 0.13	6.32 ± 0.18	6.39 ± 0.22	6.37 ± 0.16	6.35 ± 0.14
Albumin (g/dl)	4.49 ± 0.10	4.45 ± 0.009	4.54 ± 0.15	4.51 ± 0.15	4.49 ± 0.15
Bilirubin (mg/dl)	0.05 ± 0.03	0.06 ± 0.02	0.05 ± 0.02	0.07 ± 0.03	0.04 ± 0.02
Glucose (mg/dl)	238.32 ± 58.06	243.43 ± 46.93	219.21 ± 29.60	213.55 ± 63.22	219.53 ± 27.14
Uric acid (mg/dl)	4.96 ± 1.39	5.18 ± 1.18	4.70 ± 1.12	4.40 ± 1.87	4.69 ± 1.10
Triglyceride (mg/dl)	93.69 ± 24.51	95.58 ± 18.76	104.34 ± 223.74	91.39 ± 13.71	79.24 ± 12.67
Cholesterol (mg/dl)	56.54 ± 5.62	63.98 ± 11.96	55.26 ± 13.82	58.44 ± 9.15	51.80 ± 12.34
Sodium (mmol/l)	144.80 ± 2.53	144.10 ± 2.08	143.80 ± 1.69	143.60 ± 1.43	143.60 ± 1.58
Potassium (mmol/l)	7.94 ± 0.55	7.84 ± 0.96	7.55 ± 0.95	7.26 ± 1.23	8.34 ± 0.84
Chloride (mmol/l)	104.10 ± 1.29	103.30 ± 1.25	103.80 ± 1.14	104.00 ± 0.82	104.20 ± 1.62

The values are expressed as mean±SD, DW: Distilled water, TG: Tragacanth

Table 6 Biochemical values of male rats receiving AE for 90 days

Parameters	Dose of AE (mg/kg/day)				
	DW	0.5% TG	20	200	2000
	n = 10	n = 10	n = 10	n = 10	n = 10
ALP(U/l)	22.30 ± 3.71	25.00 ± 6.70	23.60 ± 3.34	26.70 ± 6.15	24.10 ± 5.80
ALT(U/l)	20.40 ± 3.17	20.90 ± 4.36	18.00 ± 1.76	18.40 ± 2.12	19.40 ± 1.84
AST(U/l)	82.40 ± 14.47	80.30 ± 6.43	78.60 ± 5.78	84.40 ± 9.83	80.30 ± 8.84
BUN (mg/dl)	23.20 ± 2.84	21.58 ± 5.94	25.71 ± 7.69	21.02 ± 3.52	20.13 ± 2.50
Creatinine (mg/dl)	0.50 ± 0.08	6.46 ± 18.81	0.57 ± 0.14	0.47 ± 0.05	0.43 ± 0.06
Total Protein (g/dl)	6.32 ± 0.15	6.17 ± 0.22	6.28 ± 0.19	6.32 ± 0.33	6.23 ± 0.16
Albumin (g/dl)	4.72 ± 0.08	4.62 ± 0.15	4.74 ± 0.15	4.68 ± 0.24	4.68 ± 0.17
Bilirubin (mg/dl)	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.06 ± 0.01
Glucose (mg/dl)	103.40 ± 16.75	121.29 ± 24.81	103.52 ± 14.00	101.46 ± 11.64	100.27 ± 10.69
Uric acid (mg/dl)	2.38 ± 0.99	2.38 ± 0.85	2.21 ± 0.65	2.08 ± 0.82	2.39 ± 0.59
Triglyceride (mg/dl)	34.35 ± 4.81	30.76 ± 5.52	34.16 ± 8.27	33.82 ± 5.05	28.07 ± 4.09
Cholesterol (mg/dl)	62.72 ± 13.03	56.39 ± 13.72	58.30 ± 14.48	58.98 ± 11.49	52.38 ± 10.44
Sodium (mmol/l)	142.50 ± 1.90	142.90 ± 1.91	142.90 ± 1.37	143.20 ± 0.79	142.80 ± 1.55
Potassium (mmol/l)	6.64 ± 1.61	6.64 ± 1.24	6.61 ± 1.57	6.51 ± 1.64	6.69 ± 1.13
Chloride (mmol/l)	106.50 ± 0.97	107.20 ± 1.40	107.30 ± 1.49	106.90 ± 0.88	106.70 ± 1.34

The values are expressed as mean±SD, DW: Distilled water, TG: Tragacanth

Table 7 Histopathological results of visceral organs of rats receiving AE for 90 days

Organs	Microscopic findings	Male					Female				
		Dose of AE (mg/kg/day)					Dose of AE (mg/kg/day)				
		DW	0.5% TG	20	200	2000	DW	0.5% TG	20	200	2000
Lung	BALT proliferation	9/10	7/10	4/10	6/10	6/10	6/10	2/10	5/10	4/10	4/10
Heart	Focal myocardiosis	0/10	0/10	0/10	0/10	1/10	NRL	NRL	NRL	NRL	NRL
Liver	Centrilobular fatty degeneration	3/10	0/10	1/10	1/10	0/10	6/10	3/10	1/10	3/10	1/10
	Centrilbular hydropic degeneration	9/10	5/10	2/10*	1/10*	3/10*	1/10	0/10	0/10	0/10	0/10
Small intestine	GALT proliferation in submucosa layer	3/10	1/10	3/10	2/10	0/10	3/10	2/10	1/10	1/10	0/10
Large intestine	GALT proliferation in submucosa layer	1/10	1/10	1/10	2/10	3/10	1/10	1/10	2/10	0/10	0/10
Adrenal gland	Cortical fatty infiltration	7/10	6/10	5/10	7/10	3/10					
	Medullary congestion						0/10	0/10	0/10	3/10	1/10
Mammary gland	Glandular hyperplasia						1/10	0/10	0/10	4/10#	2/10

The results are expressed as the number of rats with pathological findings per total number of rats treated
*significantly different from the water control group ($p < 0.05$), # significantly different from the tragacanth control group ($p < 0.05$)
DW: Distilled water, TG: Tragacanth, NRL: No remarkable lesions, BALT: Bronchiole-associated lymphoid tissue, GALT: Gut-associated lymphoid tissue

Effects of AE on histopathological alterations: At necropsy, there was no remarkable gross lesions in any visceral organs of all AE-treated and both control groups**.** Male rats receiving AE at the doses of 20, 200 and 2000 mg/kg/day had significantly lower incidence of hydropic degeneration of hepatocytes than the water control group. Female rats receiving AE at the dose of 200 mg/kg had significantly higher incidence of mammary glandular hyperplasia than the tragacanth control group. The incidence of histopathological lesions in the lung, heart, intestines, adrenal glands of the AE-treated groups revealed no significant differences when compared with those of the corresponding control groups (Table 7). No remarkable histopathological lesions was observed in other organs in both treatment and control groups.

Discussion

The total dose of AE administered to mice within 24 hours in the acute toxicity test was 5 g/kg, which did not cause any acute toxic signs, gross lesions of visceral organs and mortality. Hence, the oral LD_{50} value of AE should be more than 5 g/kg and this result indicates that AE is practically nontoxic after acute oral exposure (Chan and Hayes, 1994).

The ninety-day subchronic toxicity study in Wistar rats showed that AE at the given dose range did not affect body weight, general health status and relative organ weight. The decrease of food consumption in the highest dose-treated female group was transient and could recover in the last three weeks, therefore it may not be concluded that AE suppresses the animal food intake. Hematological results showed an increased MCHC in the male rats treated with AE 200 mg/kg/day. This phenomenon may not contribute to AE since it did not show any dose dependency. The alterations of neutrophil and eosinophil in the highest dose-treated male rats were within the normal range (Gad, 1992). In addition, hematological values in the AE-treated female rats did not show any significance. Taken together, it might be concluded that AE did not produce any abnormal changes in rat hematological values. Blood chemistry analysis in both sexes indicated that AE did not affect any examined parameters. The decrease in hepatocyte hydropic degeneration in the AE- treated male rats including the increase of female mammary glandular hyperplasia at the dose of 200 mg/kg/day may not be attributed to AE since it did not show any dose dependency. Other histopathological lesions in some organs of the males and females showed no significant difference between the treatment and the corresponding control groups, therefore these may not contribute to AE.

In conclusion, AE at the oral dose of 5 g/kg produced no acute toxic effect and mortality in mice. The ninety-day subchronic toxicity study in Wistar rats revealed that AE at the doses of 20, 200 and 2000 mg/kg/day did not produce any abnormalities in growth, food consumption, organ weights, hematological and clinical chemistry values. Furthermore, it did not cause any dose-related histopathological lesions of visceral organs. Hence, these data suggest that AE has potential for further phytopharmaceutical or health product development.

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