Original Article

Acute and Chronic Toxicity of *Moringa oleifera* Linn Leaves Extracts

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Abstract

Moringa oleifera leaves have been reported to possess potential hypotensive and hypocholesterolemic and hypoglycemic activities; nevertheless toxicological data of this herb in animal models have still been scanty. The objective of this study was to evaluate both acute and chronic toxicity of the water extract of *M. oleifera* leaves by oral administration. Acute toxicity test in mice by gavage with the extract twice, each at the dose of 10 g/kg, revealed that the extract produced no acute toxic symptoms and gross lesions of vital organs. Chronic toxicity study was investigated in eighty Wistar rats allocated into four groups, each of ten per sex. Group 1 was the control group receiving distilled water. Group 2 to 4 were experimental groups receiving the extract at the doses of 10, 100 and 1000 mg/kg/day for six months consecutively. The results revealed that the extract at different doses did not affect growth, food consumption, general health status and any hematological values of the animals. Blood chemistry profiles of the extract-treated male rats were not significantly different from those of the control-group male rats. In the female, when compared to the control group, the group receiving the extract at the dose of 100 mg/kg/day had significantly higher albumin and the highest dose–treated group had significantly lower potassium levels. Histopathological results revealed that the incidence of lesions in some organs of all extract-treated groups were not significantly different from those of the control group. In conclusion, the water extract of *M. oleifera* leaves at the tested doses produce no acute toxicity and serious chronic toxicity in experimental animals.

Keywords: *M. oleifera* leaves extract, acute, chronic toxicity, mice, rat

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บทคัดย่อ

พิษเฉียบพลันและพิษเรื้อรังของสารสกัดใบมะรุม

ทรงพล ชีวะพัฒน์[1] พรชัย สินเจริญโภไคย[1] นลินภัสร์ ศักดิ์ติยะสุนทร[1] อัศวชัย ช่วยพรม[1] ประถม ทองศรีรักษ์[1]*
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ใบมะรุมมีคุณสมบัติลดความดันเลือด ไขมันโคเลสเตอรอล และลดน้ำตาลในเลือดได้ดี แต่รายงานการศึกษาความเป็นพิษต่อสัตว์ทดลองยังมีน้อยมาก การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อให้ทราบถึงความเป็นพิษทั้งระยะเฉียบพลันและเรื้อรังของสารสกัดใบมะรุมด้วยน้ำ โดยวิธีป้อนทางปาก การทดสอบพิษเฉียบพลันในหนูถีบจักรที่ได้รับสารสกัดใบมะรุมขนาด 20 ก./กก.โดยแบ่งให้สองครั้ง ๆ ละ ขนาด 10 ก./กก. เปรียบเทียบกับกลุ่มควบคุมด้วยน้ำกลั่น แสดงให้เห็นว่า สารสกัดใบมะรุมไม่ก่อให้เกิดอาการพิษเฉียบพลันและความผิดปกติของอวัยวะสำคัญทางมหพยาธิวิทยา ในการศึกษาพิษเรื้อรังในหนูแรท พันธุ์วิสตาร์จำนวน 80 ตัว แบ่งออกเป็น 4 กลุ่ม ๆละ 20 ตัว (เพศละ 10 ตัว) ดังนี้ กลุ่มที่ 1 เป็นกลุ่มควบคุมได้รับน้ำกลั่น กลุ่มที่ 2 ถึง 4 เป็นกลุ่มทดลองที่ได้รับสารสกัดใบมะรุมขนาด 10, 100 และ 1000 มก./กก./วัน ติดต่อกันเป็นเวลา 6 เดือน ตามลำดับ ผลการทดลองพบว่า สารสกัดใบมะรุมในขนาดต่างๆ ไม่มีผลต่อการเจริญเติบโต การกินอาหาร สุขภาพทั่วไป น้ำหนักอวัยวะสัมพัทธ์ และค่าทางโลหิตวิทยา ค่าเคมีคลินิกต่างๆ ในหนูเพศผู้ที่ได้รับสารสกัดใบมะรุมไม่มีความแตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญ ในเพศเมียพบว่า กลุ่มที่ได้รับสารสกัดใบมะรุมขนาด 100 มก./กก./วัน มีค่าอัลบูมินสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ และกลุ่มที่ได้รับสารสกัดขนาด 1000 มก./กก./วัน มีระดับโปแตสเซียมในซีรั่มต่ำกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ผลการตรวจอวัยวะภายในทางจุลพยาธิวิทยา พบว่า หนูกลุ่มที่ได้รับสารสกัดใบมะรุมทั้งหมดมีอุบัติการณ์ของการเปลี่ยนแปลงแปลงในบางอวัยวะไม่แตกต่างจากหนูกลุ่มควบคุมอย่างมีนัยสำคัญ การศึกษาครั้งนี้สรุปได้ว่า สารสกัดใบมะรุมด้วยน้ำไม่ก่อให้เกิดพิษเฉียบพลันและพิษเรื้อรังที่รุนแรงต่อสัตว์ทดลอง

คำสำคัญ: สารสกัดใบมะรุม พิษเฉียบพลัน พิษเรื้อรัง หนูไมซ์ หนูแรท

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Introduction

Moringa oleifera Lam. is a short, slender and perennial tree belonging to the Moringaceae family. It is widely cultivated and naturalized in tropical India, Africa, tropical America, Sri lanka, Mexico, Malaysia and the Phillipine Islands (Sabale, 2008). The plant is used widely as antispasmodic, stimulant, expectorant, diuretics and also for the treatment of hiccough, influenza and internal abscess in Indian traditional medicines (Mishra et al., 2000). Moreover, leaves, fruit flowers and immature pods of this plant are used as a highly nutritive vegetable in many countries such as India, Pakistan, Hawaii and many parts of Africa (Sabale, 2008). In Thailand it is commonly known as "Marum" and immature fruits or pods of this plant have long been used as vegetable or as food ingredient (Wutythamawech, 1997). The leaves were proven to be a rich source of β-carotene, protein, vitamin C, calcium, potassium and antioxidant compounds such as ascorbic acid, flavonoids, carotenoids, phenolics (Sabale, 2008) and various amino acids (Mishra et al., 2000).

Biological and pharmacological studies of the compounds and the crude extract from *M.oleifera* leaves have been extensively investigated. Gilani (1994) demonstrated that pure compounds from the leaves had hypotensive and spasmolytic activities. The glycosides, niaziminins A and B and isothiocyanate isolated from the leaves extract were shown to have hypotensive effects on normotensive rats (Faizi et al., 1995). Small peptides from the aqeous extract of *M. oleifera* leaves possess antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebseilla aerogenes* and *Aspergillus niger* (Dahot, 1998). The methanol fractions of its leaf extract were reported to possess antiulcer activity and enhancement of healing process in chronic lesions (Pal et al., 1995). The water extract of *M. oleifera* leaves had a significant cholesterol lowering action in the high fat diet rats (Ghasi et al., 2000) including hypoglycemic and antidiabetic activities (Jaiswal et al., 2009). Recently, it has been demonstrated that the aqueous extract of *M. oleifera* leaves possesses antimutagenicity in Salmonella *typhimurium* strain TA100 (Charoensin and Wongpoomchai, 2010). Although many studies

have confirmed various health benefits of *M. oleifera* leaves, very little information is available regarding the toxicology of this plant. Thus, this study aimed to investigate acute and chronic toxicity of the water extract of *M. oleifera* leaves in animal models, which may be useful to assess whether the leaves are safe or possibly harmful for the employment as food supplement or health herb.

Materials and Methods

Plant material: The leaves of *M. oleifera* were collected from Chanthaburi Medicinal Plant garden of Department of Medical Sciences, Chanthaburi Province, Thailand. The plant specimens were identified by Thawatchai Wongprasert, Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation. A voucher specimen of *M. oleifera* Lam. (DMSC 5174) was deposited at the DMSC Herbarium, Medicinal Plant Research Institute, Department of Medical Science, Thailand.

Preparation of M. oleifera extract (MOE): *M. oleifera* leaves were washed, dried at 400C for 10 hours and were then pulverized into coarse powder. The dried powder was refluxed with distilled water twice, each for two hours. The solution from both extraction were pooled together and then dried using lyophilizer. The yield of the dried extract was about 19.15%. Total phenol content in the extract was determined using the method modified from Folin Ciocalteu (Obanda and Owvor, 1997). It was found that the total polyphenol content was equal to 53.7 mg gallic acid equivalents/g extract. The extract was suspended in distilled water and was adjusted for the differently desired concentration for further acute and chronic toxicity study in mice and rats, respectively.

Animals: Twenty ICR mice, (10 males and 10 females), each weighing 20-22 g, and eighty Wistar rats, 40 males weighing 180-200 g and 40 females weighing 170-190 g, were purchased from The National Laboratory Animal Center, Mahidol University. The animals were housed in strict hygienic conventional mice and rat rooms at the laboratory animal center, Department of Medical Sciences where the environment of the room was maintained at 25±1^{0}C 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 the acute toxicity testing. Prior to the chronic toxicity study, the rats were acclimatized with the environment for two weeks. This study was approved by the Institutional Animal Care and Use Committee, Department of Medical Sciences (Approval No. 52-031)

Acute toxicity test: The mice were randomly divided into two groups, each of ten animals (five male and five female). The experimental group was orally given MOE suspension at dose of 10 g/kg and observed for five hours. The process was then repeated with an equal dose. The control group was given distilled water at the volume of 20 ml/kg twice. 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.

Chronic toxicity study: The wistar rats were randomly allocated to four groups of ten animals of each sex. Group 1 was the control group receiving distilled water at the volume of 10 ml/kg. Group 2 to 4 were treatment groups orally administered with MOE at the doses of 10, 100, and 1000 mg/kg /day for six months, which were approximately equivalent to 1, 10 and 100 times of the dried Marum leaf used in human food supplement, respectively. 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 six-month treatment period, the animals were fasted overnight and 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 (Hb), erythrocyte (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), neutrophils, eosinophils, lymphocytes, monocytes, basophils and platelets. 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. The 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 weight was calculated into relative organ weight (g/1000 g body weight). 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 gland, 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 testing: Mice receiving MOE extract at dose of 10.0 g/kg twice did not manifest any clinical signs or behaviors during the observation period when compared to the control group. All MOE-treated mice survived until the end of the experiment. Necropsy revealed no gross lesions of their visceral organs when compared with the control group.

Chronic toxicity study:

Effect of MOE on body weight, food consumption health status and relative organ weights: Both male and female rats receiving MOE at different doses showed no significant difference in their average body weight and food consumption when compared with the corresponding control group over the whole experimental period (Figs 1 and 2). All of the MOE-treated groups revealed healthy and showed no clinically toxic signs, as compared to the control group. Relative organ weights of all MOE-treated groups were not significantly different from those of the control group (Table 1).

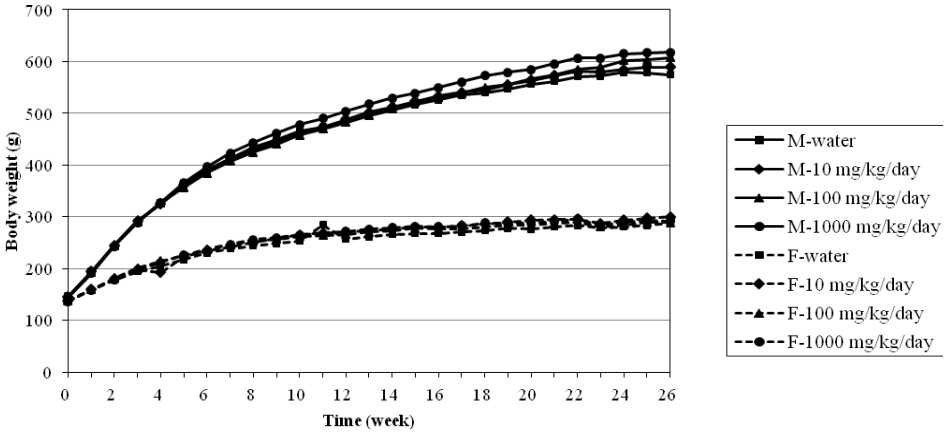

Figure 1 Growth curves of male (M) and female (F) rats receiving MOE for 6 months.

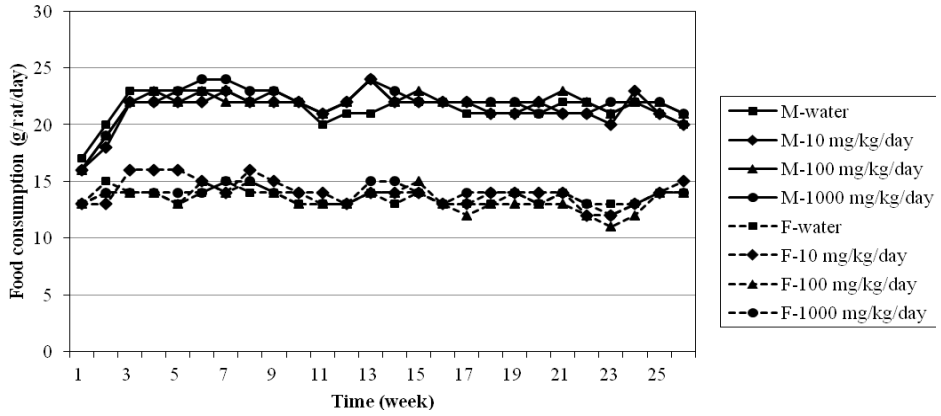

Figure 2 Food consumption of male (M) and female (F) rats receiving MOE for 6 months

Effect of ME on hematological and biochemical value: As shown in table 2 there were no significant differences in any hematological values between all MOE-treated and control groups. Blood chemistry profiles showed no significant differences in almost all of the studied parameters between the treatment and control groups. Except in the females, the group receiving 100 mg/kg/day of MOE had significantly higher albumin and the highest dose-treated group had significantly lower potassium level than their corresponding control group (Table 3).

E***ffects of MOE on histopathological lesions:*** At necropsy, there was no remarkable macroscopic lesion in any organs of both MOE-treated and control groups. Except a male rat receiving the extract at dose of 100 mg/kg had splenomegaly caused by splenic lymphoma confirmed by histopathology. The incidence of histopathological lesions in the lung, heart, liver, kidney, intestines, adrenal glands of both sexes and female mammary glands of the MOE-treated groups revealed no significant differences when compared with those of the corresponding control group (Table 4). Furthermore, there were no remarkable histopathological changes in the other organs in both treatment and control groups.

Table 1 Relative organs weight (g/1000 g body weight) of male and female rats receiving MOE for 6 month

Organs	Male				Female			
	Dose of MOE (mg/kg/day)				Dose of MOE (mg/kg/day)			
	0	10	100	1000	0	10	100	1000
	n=10	n=10	n=9#	n=10	n=10	n=10	n=10	n=10
Brain	3.95±0.38	3.84±0.30	3.90±0.56	3.67±0.45	7.15±0.74	6.83±0.61	7.03±0.58	7.15±0.55
Heart	2.72±0.19	2.59±0.20	2.70±0.26	2.63±0.20	3.35±0.29	3.30±0.17	3.34±0.26	3.35±0.21
Lung	3.34±0.23	3.12±0.24	3.21 ± 0.37	3.12±0.38	4.46±0.44	4.44±0.53	4.43±0.46	4.30±0.32
Liver	25.59±2.25	25.32±1.74	26.30±2.60	26.21±1.64	26.02±2.60	26.12±5.90	27.53±2.42	27.48±1.46
Stomach	4.07±0.33	3.96±0.56	4.12±0.55	3.77±0.53	5.73±0.56	5.56±0.71	5.82±0.54	5.58±0.77
Spleen	1.62±0.21	1.69±0.16	1.72±0.29	1.61±0.19	2.27±0.28	2.24±0.23	2.39±0.21	2.04±0.18
Rt Kidney	2.46±0.24	2.41±0.22	2.46±0.29	2.37±0.24	3.00±0.19	2.93±0.20	2.96±0.30	3.02±0.24
Lt Kidney	2.40±0.24	2.30±0.20	2.32±0.23	2.19±0.20	2.80±0.30	2.71±0.17	2.75±0.27	2.82±0.20
Rt Adrenal	0.07±0.01	0.07±0.02	0.07±0.02	0.06±0.01	0.17±0.03	0.15±0.03	0.16±0.03	0.17±0.03
Lt Adrenal	0.07±0.02	0.07±0.02	0.08±0.01	0.07±0.01	0.18±0.02	0.17±0.03	0.16±0.02	0.18±0.02
Bladder	0.29±0.03	0.28±0.05	0.29±0.04	0.27±0.06	0.32±0.05	0.30±0.05	0.30±0.08	0.31±0.06
Rt Testis	5.26±0.72	5.32±0.66	5.24±0.68	4.70±0.87				
Lt Testis	5.38±0.73	5.32±0.56	5.32±0.68	4.84±1.02				
Uterus					2.28±0.69	2.45±0.53	2.43±1.10	2.11±0.49
Rt Ovary					0.30±0.09	0.26±0.07	0.25±0.06	0.30±0.08
Lt Ovary					0.29±0.07	0.28±0.06	0.30±0.08	0.33±0.10

Data shown were mean±SD. #Autopsy revealed one male rat with splenic lymphoma

Table 2 Hematological values of male and female rats receiving *Moringa oleifera* extract (MOE) for 6 months.

Parameters	Male				Female			
	Dose of MOE (mg/kg BW/day)				Dose of MOE (mg/kg BW/day)			
	0	10	100	1000	0	10	100	1000
	n=10	n=10	n=9#	n=10	n=10	n=10	n=10	n=10
Hematocrit (%)	31.58 ± 1.43	32.02 ± 1.54	31.38 ± 2.09	30.38 ± 0.86	32.64 ± 1.63	33.68 ± 2.52	32.41 ± 2.49	31.07 ± 1.26
Hemoglobin (g/dl)	16.26 ± 0.77	16.56 ± 0.68	16.13 ± 1.09	15.55 ± 0.35	16.93 ± 0.82	17.47 ± 1.22	16.76 ± 1.22	16.18 ± 0.48
RBC ($x 10^6$ cells/mm^3)	9.26 ± 0.37	9.50 ± 0.51	9.33 ± 0.71	9.06 ± 0.21	9.06 ± 0.49	9.24 ± 0.84	8.78 ± 0.77	8.61 ± 0.27
MCV (fl)	34.11 ± 0.68	33.76 ± 0.62	33.67 ± 0.76	33.54 ± 0.66	36.06 ± 0.65	36.50 ± 1.00	36.94 ± 1.30	36.08 ± 0.66
MCH (pg)	17.57 ± 0.33	17.44 ± 0.44	17.31 ± 0.39	17.18 ± 0.31	18.70 ± 0.38	18.94 ± 0.64	19.10 ± 0.61	18.79 ± 0.27
MCHC (g/dl)	51.50 ± 0.48	51.69 ± 0.74	51.43 ± 0.74	51.19 ± 0.77	51.87 ± 0.30	51.88 ± 0.64	51.73 ± 0.46	52.08 ± 0.99
WBC ($x 10^3$ cells/mm^3)	3.37 ± 0.45	3.38 ± 0.90	2.96 ± 0.90	3.58 ± 1.56	2.19 ± 0.54	2.24 ± 1.06	2.42 ± 0.51	1.73 ± 0.31
Neutrophil (%)	22.67 ± 4.38	26.74 ± 8.00	27.23 ± 6.28	31.95 ± 13.27	25.90 ± 4.77	29.23 ± 9.60	26.73 ± 5.34	26.83 ± 3.75
Eosinophil (%)	1.54 ± 0.95	1.64 ± 0.43	1.70 ± 0.68	1.59 ± 0.76	1.85 ± 0.61	1.31 ± 0.49	1.52 ± 0.58	2.37 ± 2.13
Lymphocyte (%)	71.51 ± 6.62	67.80 ± 8.44	66.06 ± 9.37	61.23 ± 12.94	69.97 ± 5.59	66.41 ± 9.28	67.42 ± 6.01	66.26 ± 6.74
Monocyte (%)	3.12 ± 2.51	2.76 ± 2.99	3.67 ± 3.32	3.45 ± 2.88	1.85 ± 1.74	2.44 ± 2.17	3.39 ± 3.46	3.59 ± 3.37
Basophil (%)	1.16 ± 1.19	1.05 ± 1.14	1.32 ± 1.51	1.78 ± 1.74	0.44 ± 0.65	0.61 ± 0.65	0.95 ± 0.73	0.95 ± 0.98
Platelet ($x 10^3$ cells/mm^3)	962.60 ± 76.68	976.10 ± 120.91	957.72 ± 98.32	960.65 ± 63.02	931.95 ± 80.74	960.30 ± 90.95	959.25 ± 88.08	921.55 ± 98.96

Data shown were mean±SD.
#Autopsy revealed one male rat with splenic lymphoma

Data shown were mean±SD.
#Autopsy revealed one male rat with splenic lymphoma

Table 3 Clinical chemistry values of male and female rats receiving *Moringa oleifera* extract (MOE) for 6 months.

Parameters	Male				Female			
	Dose of MOE (mg/kg/day)				Dose of MOE (mg/kg/day)			
	0	10	100	1000	0	10	100	1000
	n=10	n=10	n=9#	n=10	n=10	n=10	n=10	n=10
ALP(U/l)	46.60 ± 4.70	51.30 ± 8.94	49.78 ± 7.69	50.80 ± 8.87	25.60 ± 8.03	22.30 ± 3.50	25.00 ± 5.19	21.70 ± 6.17
ALT(U/l)	38.60 ± 12.29	47.40 ± 22.59	34.78 ± 12.25	38.30 ± 6.88	23.50 ± 5.40	30.30 ± 14.35	27.90 ± 6.14	28.50 ± 10.50
AST(U/l)	90.20 ± 17.87	96.80 ± 27.60	102.56 ± 74.88	84.20 ± 6.78	75.10 ± 9.30	81.10 ± 15.78	82.80 ± 11.24	79.80 ± 9.15
BUN (mg/dl)	18.05 ± 1.68	16.66 ± 2.11	18.71 ± 5.17	16.03 ± 2.07	22.00 ± 2.88	20.59 ± 2.78	20.91 ± 3.02	22.24 ± 2.91
Creatinine (mg/dl)	0.53 ± 0.05	0.49 ± 0.03	0.55 ± 0.09	0.51 ± 0.07	0.48 ± 0.03	0.47 ± 0.05	0.50 ± 0.09	0.51 ± 0.04
Total Protein (g/dl)	6.61 ± 0.27	6.59 ± 0.21	6.41 ± 0.20	6.56 ± 0.22	6.27 ± 0.31	6.53 ± 0.34	6.55 ± 0.39	6.37 ± 0.36
Albumin (g/dl)	4.51 ± 0.11	4.52 ± 0.10	4.43 ± 0.14	4.43 ± 0.14	4.77 ± 0.18	4.86 ± 0.24	4.95 ± 0.26*	4.81 ± 0.24
Bilirubin (mg/dl)	0.08 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.11 ± 0.02	0.08 ± 0.02
Glucose (mg/dl)	279.60 ± 47.61	285.37 ± 50.94	256.41 ± 70.79	271.91 ± 90.10	119.56 ± 33.14	115.81 ± 21.93	113.43 ± 21.95	111.95 ± 18.13
Uric acid (mg/dl)	5.58 ± 1.11	6.59 ± 1.08	5.68 ± 1.38	5.92 ± 1.96	2.32 ± 0.50	2.76 ± 0.49	2.65 ± 0.44	2.06 ± 0.38
Triglyceride (mg/dl)	71.68 ± 25.36	83.29 ± 35.74	93.69 ± 38.44	96.20 ± 61.54	37.80 ± 5.61	38.32 ± 4.90	44.90 ± 14.38	43.19 ± 14.15
Cholesterol (mg/dl)	66.53 ± 16.98	68.22 ± 11.60	69.93 ± 12.85	79.98 ± 19.77	65.48 ± 16.34	69.70 ± 10.67	69.37 ± 21.30	63.26 ± 17.92
Na^+ (mmol/l)	144.90 ± 1.10	144.10 ± 1.52	144.11 ± 1.05	144.70 ± 1.42	142.10 ± 0.99	142.70 ± 1.25	142.40 ± 1.26	142.90 ± 0.88
K^+ (mmol/l)	7.54 ± 0.34	7.20 ± 0.44	7.79 ± 0.80	7.23 ± 0.89	8.00 ± 0.66	7.83 ± 0.57	7.56 ± 0.46	7.21 ± 0.66*
Cl^- (mmol/l)	104.40 ± 1.35	104.40 ± 1.07	104.67 ± 0.87	104.70 ± 1.16	106.50 ± 1.27	106.60 ± 1.71	106.10 ± 0.88	107.90 ± 1.29

Data shown were mean±SD.
*Significantly different from control group ($p<0.05$)
#Autopsy revealed one male rat with splenic lymphoma

Table 4 Histopathological results of male and female rats receiving MOE for 6 months.

Organs	Microscopic findings	Male				Female			
		Dose of MOE (mg/kg /day)				Dose of MOE (mg/kg /day)			
		0	10	100	1000	0	10	100	1000
Lung	BALT proliferation	8/10	8/10	9/10	4/10	6/10	7/10	5/10	5/10
Heart	Focal myocardiosis	0/10	0/10	0/10	1/10	NRL	NRL	NRL	NRL
Liver	Centrilobular fatty degeneratian	2/10	4/10	4/10	2/10	2/10	1/10	0/10	1/10
	Centrilbular hydropic degeneration	3/10	1/10	0/10	4/10	0/10	0/10	0/10	0/10
	Bile ductule proliferation	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10
Spleen	Lymphoma	0/10	0/10	1/10	0/10	NRL	NRL	NRL	NRL
Kidney	Dilated tubule	0/10	1/10	1/10	1/10	0/10	0/10	0/10	0/10
	Chronic interstitial nephritis	0/10	0/10	1/10	1/10	NRL	NRL	NRL	NRL
Small intestine	GALT proliferation in submucosa	NRL	NRL	NRL	NRL	1/10	1/10	0/10	0/10
Large intestine	GALT proliferation in submucosa	2/10	2/10	1/10	2/10	1/10	1/10	2/10	0/10
Adrenal gland	Cortical fatty infiltration	4/10	5/10	7/10	8/10	NRL	NRL	NRL	NRL
Mammary gland	Glandular hyperplasia					2/10	3/10	5/10	2/10

Data shown were number of rats with histopathological lesions / total number of rat in each group
(NRL: No remarkable lesions, BALT: Bronchiole-associated lymphoid tissue, GALT : Gut-associated lymphoid tissue)

Discussion

In acute toxicity study, total dose of MOE administered to mice within 24 hours was 20 g/kg which do not cause any acute toxic signs, gross lesions of visceral organs and induced mortality rate. Hence, the median lethal dose (LD50) of MOE should be more than 20 g/kg and this given dose was approximately 500 times higher than dried Marum leaf powder used in human food supplement (~40 mg/kg/day).

Chronic toxicity study in Wistar rats showed that MOE did not affect body weight, food consumption, general health status, relative organ weight and hematological parameters values in both sexes. Blood chemistry analysis suggested that MOE did not cause any significant changes in almost all of the examined parameters. A significant increase in albumin in the female rats treated with MOE at the dose of 100 mg/kg did not show any dose dependency and was still within the rat reference range (Pimainog et al, 2003). Therefore, it may not be caused by MOE. However, the significant decrease in serum potassium in the female rats receiving the highest dose of MOE might contribute to MOE effects since it tended to show dose dependency. However, this group of rats did not show any abnormal signs related to hypokalemia status such as muscular weakness. Caceres et al. (1994) demonstrated that the hot water infusion of M. oleifera leaves exerted diuretic activity in the rats. Thus, MOE may possibly act as a potassium-losing diuretic and result in the decreased potassium level, which needs further investigation. The incidence of histopathological lesions in some organs showed no significant differences between the MOE-treated and control groups of both sexes. In addition, no remarkable lesion was found in other studied organs. Taken together, these findings may be indicative that MOE does not induce any histological lesions in various visceral organs. The finding of splenic lymphoma in a male rat receiving MOE at dose of 100 mg/kg may not be attributable to MOE since this finding was not found in any of the MOE-treated groups. Moreover, this type of tumor was also found in histopathological study in control laboratory rats (Peckham, 1995)

In conclusion, MOE produced no acute toxic effect and had LD50 value in mice more than 20 g/kg. The chronic toxicity study in Wistar rats revealed that MOE at different doses did not affect growth, food consumption, organ weight, hematological values and almost all of clinical chemistry values. In addition, MOE did not cause any abnormalities in the studied visceral organs. However, the decrease in potassium level in the female rats receiving the highest dose of MOE suggest that the long term consumption of high dose of MOE may affect potassium level, and therefore it may not be suitable for patients with hypokalemia and arrhythmia.

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