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Original Article

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Abstract

Ethanolic extracts from fruit pericarp of mangosteen (*Garcinia mangostana* L.) possess many biological and pharmacological activities. However, chronic toxicity study of ethanolic extract has never been investigated. The objective of this study was to evaluate the safety of 95% ethanolic extract from mangosteen pericarp in animal model. The oral administration of the extract was performed in 180 Wistar rats randomly allocated to six groups, each of 15/sex. Group 1 was control group receiving distilled water. Group 2 to 6 were treatment groups receiving the extract at the doses of 10, 100, 500, 1000 and 1000 mg/kg/day for six months respectively. The last group was assigned to be the satellite group for the study of reversibility of the extract effects after two-week of extract withdrawal. The results revealed that the highest dose extract produced significantly lower body weights in both male and female rats, compared to their corresponding control groups. The extract at any tested doses did not affect the animals' behavior, health status and nor did produce any abnormality of clinical manifestations and hematological values. Clinical chemistry results showed that the male rats treated with 500 mg/kg/day extract onward had significantly higher ALT than their control group. Both male and female receiving the highest dose extract had significantly higher AST, whereas their glucose levels were significantly lower when compared to their corresponding control groups. The male rats of the highest dose and satellite groups had significantly higher BUN values than their control group. The female rats receiving the extract at the dose of 500 mg/kg/day onward had significantly higher BUN and creatinine values than their control group. Histopathological results of visceral organs revealed no significant lesion related to the extract; except the satellite group of both sexes, which had significantly higher lesion of centrilobular hydropic degeneration in their livers than the corresponding control groups. Such alteration may be caused by the highest dose mangosteen pericarp extract. In conclusion, the high dose mangosteen pericarp extract affected liver and kidney. Safety of chemical constituents in the extract should be further investigated before the usage for health promotion.

Keywords: chronic toxicity, mangosteen pericarp extract, rat

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บทคัดย่อ

การศึกษาพิษเรื้อรังของสารสกัดเปลือกมังคุด

ทรงพล ชีวะพัฒน์ [1] ปราณี ชวลิตธำรง [1] ประไพ วงศ์สินคงมั่น [1] ชฎา พิศาลพงศ์ [2] อนุเทพ รังสีพิพัฒน์ [3]*

สารสกัดเปลือกมังคุดมีฤทธิ์ทางชีวภาพและเภสัชวิทยาที่น่าสนใจหลายประการ แต่ยังไม่มีรายงานการศึกษาพิษเรื้อรัง การศึกษาครั้งนี้มีวัตถุประสงค์ เพื่อให้ทราบถึงความปลอดภัยของสารสกัดเปลือกมังคุดด้วยเอทานอล โดยวิธีป้อนสารสกัดทางปากแก่หนูแรทพันธุ์วิสตาร์ จำนวน 180 ตัว แบ่งออกเป็น 6 กลุ่มๆละ 30 ตัว (เพศละ 15 ตัว) ดังนี้ กลุ่มที่ 1 กลุ่มควบคุมได้รับน้ำกลั่น กลุ่มที่ 2 ถึง 6 เป็นกลุ่มทดลอง ที่ได้รับสารสกัดเปลือกมังคุดขนาด 10, 100, 500,1000 และ 1000 มก./กก./วัน เป็นเวลา 6 เดือนตามลำดับ โดยกลุ่มสุดท้ายเป็นกลุ่มศึกษาผลย้อนกลับ (satellite group) ภายหลังหยุดให้สารสกัดเป็นเวลา 2 สัปดาห์ ผลการศึกษาพบว่า สารสกัดเปลือกมังคุดขนาด 1000 มก./กก./วัน ทำให้หนูเพศผู้และเพศเมีย มีน้ำหนักตัวต่ำกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) สารสกัดทุกขนาดไม่มีผลต่อพฤติกรรม สุขภาพ รวมทั้งไม่ทำให้หนูมีอาการแสดงออกและค่าทางโลหิตวิทยาผิดปกติแต่อย่างใด การตรวจค่าทางเคมีคลินิก พบว่า หนูเพศผู้ที่ได้รับสารสกัดตั้งแต่ 500 มก./กก./วันขึ้นไป มีค่าเอนไซม์ ALT สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) หนูเพศผู้และเพศเมียที่ได้รับสารสกัดขนาด 1000 มก./กก./วัน มีเอนไซม์ AST สูงขึ้นอย่างมีนัยสำคัญ (p<0.05) แต่มีระดับกลูโคสลดลงอย่างมีนัยสำคัญ (p<0.05) เมื่อเปรียบเทียบกับกลุ่มควบคุม หนูเพศผู้ที่ได้รับสารสกัดขนาด 1000 มก./กก./วัน และกลุ่ม satellite มีค่า BUN สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) ส่วนหนูเพศเมียกลุ่มที่ได้รับสารสกัดตั้งแต่ 500 มก./กก./วัน ขึ้นไปมีค่า BUN และ creatinine สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) ผลทางจุลพยาธิวิทยาของอวัยวะภายใน ไม่พบรอยโรคใดๆ ที่เกิดจากสารสกัดเปลือกมังคุด ยกเว้นหนูกลุ่ม satellite พบรอยโรคการเสื่อมแบบมีน้ำในเซลล์ตับสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (p<0.05) ซึ่งอาจเกิดจากสารสกัดเปลือกมังคุดขนาดสูงสุด การศึกษาครั้งนี้สรุปได้ว่า สารสกัดเปลือกมังคุดขนาดสูงมีผลต่อตับและไต หากนำไปใช้เสริมสุขภาพควรมีการศึกษาด้านความปลอดภัยขององค์ประกอบทางเคมีต่างๆเพิ่มเติมต่อไป

คำสำคัญ: พิษเรื้อรัง สารสกัดเปลือกมังคุด หนูแรท

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Introduction

Fruit pericarp of mangosteen (*Garcinia mangostana* L.) has been traditionally used for centuries in Southeast Asians as a medicinal agent for the treatment of skin infections, wounds, amoebic dysentery and also inflammation, diarrhea, cholera and dysentery in Ayurvedic medicine. (Pedraza-Chaverri et al., 2008). Several phytochemical studies have shown that there are many xanthones compounds in the pericarp of mangosteen fruit. For example, α- mangostin, γ-mangostin, 8-deoxygartnin, garcinone E, mangostanol (Chairungsrilerd et al., 1996), tovophillin A and B (Huang et al., 2001), mangostenin (Suksamrarn et al., 2003), mangotenones C, D and E (Suksamrarn et al., 2006).

Many biological and pharmacological activities of the compounds extracted from mangosteen pericarp including its crude extracts have been extensively investigated. For instance, α-mangostin, the most abundant compound in the pericarp extract, exert antiproliferative activity against human leukemia cells (Matsumoto et al., 2003) and also possess antimalarial properties (Mahabusarakam et al., 2006). Moreover, this compound was shown to possess potent chemopreventive effects in rat colon carcinogenesis (Nabandith et al., 2004). Garcinone E has potent cytotoxic effect on lung, gastric, lung cancer human cell lines (Ho et al., 2002). Suksamrarn et al. (2003) has shown that α-and β-mangostin and garcinone B exhibit strong antituberculosis activity. An ethanolic extract of the mangosteen pericarp was demonstrated to possess antibacterial (Voravuthikunchai and Kitipat, 2005), antioxidant and neuroprotective (Weecharangsan et al., 2005), antiallergy (Nakatani et al., 2002) and anti-inflammatory activities in experimental animals (Reanmongkol and Wattanapiromkul, 2008). In

addition, it has been reported to exert the remarkable activity against SKBR3 human breast adenoma cell line (Moongkarndi et al., 2004).

Even though mangosteen pericarp has been shown to possess various health benefits, the long-term toxic effect of its extract has never been reported. In this study, we investigated chronic toxicity of the mangosteen pericarp extract in experimental animal to gain additional safety information. The results will be beneficial for supporting the development and the consumption of health products from mangosteen pericarp.

Materials and Methods

Plant material and preparation of mangosteen pericarp extract (MPE): The fruits of *G. mangostana* were purchased from Chantaburi Province, Thailand. The voucher specimen (WGM0615) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Thailand. The dried pericarp of mangosteen fruit was coarsely pulverized into powder. The powder was macerated twice with 95% ethanol for 48 and 24 hours respectively. The extract solution from each marceration was filtered and concentrated by evaporation under reduced pressure. Both concentrated extracts were pooled together and heated at 50ºC to remove the solvent. The upper part of the concentrated extract was further evaporated with rotary evaporator at 55ºC under reduced pressure, and dried with vacuum oven at 50ºC for 12 hours. The lower part of the concentrated extract was centrifuged with high-speed centrifuge at 9,000 rpm for ten min, then the supernatant solution was evaporated at 55ºC under reduced pressure using rotary evaporator and then was subjected to vacuum oven at 50ºC for 12 hours. Both concentrated extracts were mixed together at 55ºC until dried. The yield of dried MPE from the dried mangosteen pericarp was about 10% (w/w). Alpha-mangostin, a major biological active compound in MPE, was found to be 24.42% according to HPLC analysis. In addition, total tannins content in MPE was assayed by the applied determination of tannins method described in Thai Herbal Pharmacopoeia Vol II (Department of Medical Sciences, 2000) and it was found to be 13.8% (w/w). The extract was kept in well-closed container, protected from light at -20ºC for further toxicological investigation.

Animals: One hundred and eighty Wistar rats (90 male and 90 female rats weighing approximately 180-200 and 170-190 g, respectively) were purchased from The National Laboratory Animal Center, Mahidol University. 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±1ºC with 60% humidity and 12 hour-light-dark cycle. They were raised with commercial pellet diet and clean water *ad lib*. 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. 49-011).

Chronic toxicity test: Wistar rats were randomly allocated to six groups of fifteen animals of each sex. Group1 was control group receiving distilled water at the volume of 10 ml/kg. Group 2 to 6 were experimental groups orally administered with MPE at the doses of 10, 100, 500, 1000 and 1000 mg/kg/day for six months respectively. The last group (satellite group) was further raised without treatment for 14 days, in order to assess reversibility of adverse effects which may be produced by the highest dose extract. 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, 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 red blood cells (RBC), hematocrit (Hct), hemoglobin, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cells (WBC), neutrophils, eosinophils, lymphocytes, monocytes, basophils and platelets. Clinical chemistry values were measured by using automatic chemistry analyzer Hitachi® 912 (Hitachi Ltd, Japan) 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 pathological alterations 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 Int Inc, Switzerland). The organs' 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 the trachea, lymph nodes, esophagus, pancreas, intestine, 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 group were made by Bonferroni test. For histopathological results, Fisher's exact was applied. Differences between groups were considered significant at $p<0.05$.

Results

Effect of MPE on body weight, food consumption and health status: Male rats receiving MPE at the doses of 1000 and 500 mg/kg/day had significantly lower average body weight than their control group since

the 7th and 17th week till the end of the study respectively. Similar body weight change was observed in the female rats treated with the highest dose MPE at the 12th week onward (Fig. 1). Measurement of the weekly food intake in the male and female over the whole experimental period showed no significant difference between all the treatment groups and their corresponding control groups in almost every week. Only the male and female rats receiving the highest dose had significantly lower food intake than their corresponding control groups at week 8, 9 and 14 in the former group and at week 2 in the latter group (Fig. 2). All of the MPE-treated groups revealed healthy and no sign of abnormality, as compared to their control groups.

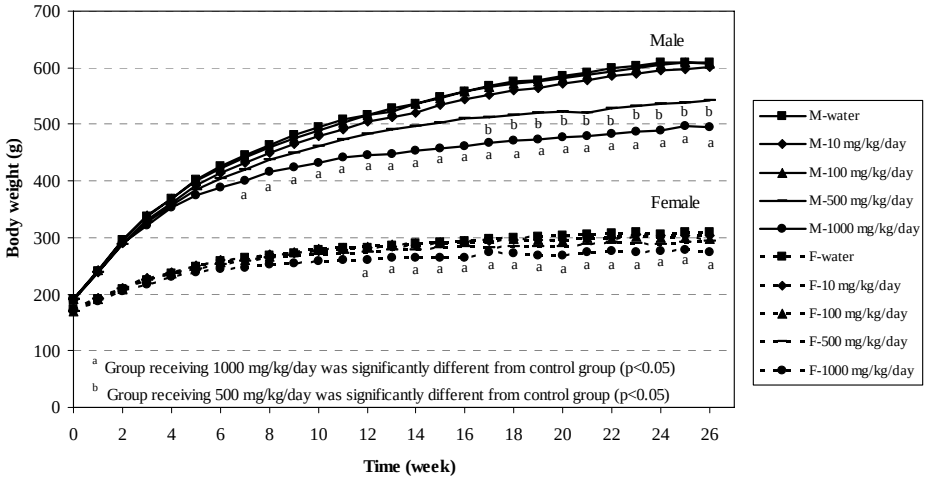

Figure 1 Growth curves of male and female rats receiving MPE for 6 months.

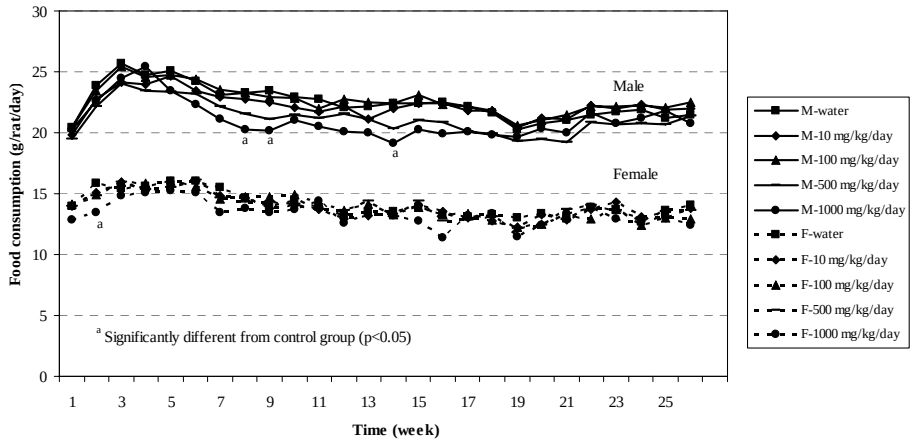

Figure 2 Food consumption of male and female rats receiving MPE for 6 months.

Table 1 Relative organ weight (g/1000 g of body weight) and body weight (g) of male rats receiving MPE for 6 months

Organs	Dose of MPE administered (mg/kg/day)					
	Control	10	100	500	1000	1000-S
	n =15	n =15	n =15	n =15	n =15	n =15
Brain	3.70 ± 0.36	3.87 ± 0.39	3.71 ± 0.36	4.22 ± 0.32*	4.65 ± 0.36*	4.54 ± 0.26*
Heart	2.55 ± 0.26	2.71 ± 0.17	2.56 ± 0.15	2.73 ± 0.16	2.89 ± 0.15*	2.90 ± 0.18*
Lung	3.01 ± 0.38	3.25 ± 0.35	3.14 ± 0.32	3.50 ± 0.38*	3.76 ± 0.44*	3.52 ± 0.24*
Liver	26.63 ± 2.97	27.85 ± 2.01	27.79 ± 2.29	28.78 ± 1.69	30.14 ± 2.69*	30.24 ± 2.98*
Stomach	3.76 ± 0.39	4.03 ± 0.44	3.91 ± 0.26	4.62 ± 0.46*	5.57 ± 0.88*	4.75 ± 0.49*
Spleen	1.51 ± 0.20	1.68 ± 0.27	1.60 ± 0.15	1.74 ± 0.21	1.77 ± 0.23*	1.86 ± 0.23*
Right kidney	2.29 ± 0.26	2.37 ± 0.17	2.36 ± 0.14	2.72 ± 0.25*	2.79 ± 0.24*	2.86 ± 0.23*
Left kidney	2.20 ± 0.22	2.27 ± 0.16	2.26 ± 0.17	2.55 ± 0.18*	2.71 ± 0.24*	2.76 ± 0.23*
Right testis	4.96 ± 0.40	5.13 ± 0.68	4.94 ± 0.64	5.78 ± 0.74*	5.86 ± 1.18*	6.12 ± 0.54*
Left testis	4.97 ± 0.40	5.30 ± 0.72	5.01 ± 0.76	5.79 ± 0.86	5.98 ± 1.16*	6.16 ± 0.54*
Right adrenal	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01*	0.06 ± 0.01
Left adrenal	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.01*	0.07 ± 0.01
Bladder	0.27 ±0.05	0.28 ± 0.07	0.27 ± 0.05	0.27 ± 0.05	0.29 ± 0.06	0.28 ± 0.05
Initial body weight	190.59±9.56	188.85±8.75	191.50±6.72	191.01±11.73	191.39±9.95	189.25±12.29
Final body weight	592.41±52.99	577.43±57.57	594.12±60.61	519.09±46.54*	465.91±37.80*	480.20±32.36*

The values are expressed as mean±SD, 1000-S: the satellite group
*significantly different from control group (p<0.05)

Effect of ME on relative organ weight: In the male, relative weight of the brain, lung, stomach, both kidneys and right testis in the group receiving 500 mg/kg/day of MPE were significantly higher than those in the control group. Almost all organs in the highest dose group had significantly higher relative weight than those of their control groups except bladder. Similar changes were observed in the satellite group except for adrenal glands and bladder (Table 1). In the female rat, relative stomach weight in the groups treated with 500 mg/kg/day MPE onward was significantly higher than that in the control group. The relative weight of the liver and both kidneys in the highest dose and satellite groups were significantly higher than those in the control group. In addition, the highest dose group had significantly higher relative brain weight than the control group (Table 2).

Table 2 Relative organ weight (g/1000g body weight) and body weight (g) of female rats receiving MPE for 6 months

Organs	Dose of MPE administered (mg/kg/day)					
	Control	10	100	500	1000	1000-S
	n =15	n =15	N =15	n =15	n =15	n =15
Brain	6.75 ± 0.40	6.88 ± 0.59	7.13 ± 0.51	7.20 ± 0.40	7.78 ± 0.52*	7.25 ± 0.45
Heart	3.21 ± 0.40	3.27 ± 0.26	3.26 ± 0.25	3.29 ± 0.22	3.47 ± 0.26	3.36 ± 0.24
Lung	4.23 ± 0.44	4.21 ± 0.67	4.59 ± 0.49	4.55 ± 0.42	4.58 ± 0.31	4.55 ± 0.40
Liver	26.89 ± 3.09	27.28 ± 2.68	26.74 ± 2.80	27.46 ± 2.13	30.31 ± 2.24*	30.72 ± 2.46*
Stomach	5.46 ± 0.59	5.79 ± 0.63	5.88 ± 0.65	6.29 ± 0.54*	7.70 ± 0.97*	6.75 ± 0.71*
Spleen	2.04 ± 0.56	2.25 ± 0.82	2.02 ± 0.28	2.01 ± 0.22	2.15 ± 0.27	2.04 ± 0.29
Right kidney	2.82 ± 0.22	2.91 ± 0.35	2.89 ± 0.23	3.05 ± 0.27	3.22 ± 0.23*	3.18 ± 0.30*
Left kidney	2.67 ± 0.13	2.74 ± 0.26	2.78 ± 0.25	2.87 ± 0.22	3.04 ± 0.15*	3.05 ± 0.23*
Right adrenal	0.12 ± 0.04	0.13 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.23 ± 0.38
Left adrenal	0.14 ± 0.03	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	0.24 ± 0.41
Bladder	0.31 ± 0.09	0.32 ± 0.06	0.29 ± 0.05	0.31 ± 0.06	0.34 ± 0.04	0.33 ± 0.03
Uterus	2.08 ± 0.79	2.52 ± 0.90	2.34 ± 0.44	2.40 ± 0.84	2.38 ± 0.52	2.50 ± 0.65
Right ovary	0.24 ± 0.09	0.25 ± 0.06	0.24 ± 0.06	0.27 ± 0.08	0.25 ± 0.08	0.43 ± 0.74
Left ovary	0.25 ± 0.09	0.26 ± 0.07	0.25 ± 0.08	0.27 ± 0.10	0.25 ± 0.06	0.25 ± 0.06
Initial body weight	173.61±8.38	174.36±7.77	169.73±8.50	169.53±6.83	169.47±5.52	171.69±11.11
Final body weight	295.19±25.60	289.49±26.22	283.63±15.18	275.68±14.74	252.75±18.20*	272.97±16.07*

The values are expressed as mean±SD, 1000-S: the satellite group
*significantly different from control group (p<0.05)

Effects of ME on hematological values: As depicted in Table 3 and 4, eosinophils in both male and female rats receiving MPE at the doses of 500 and 1000 mg/kg/day were significantly lower than those in their corresponding control groups. Neutrophils in the male rats of highest dose and satellite groups were significantly higher than those in the control group. In addition, WBC in the female rats of the satellite group was significantly higher than that in the control group.

Effects of ME on clinical chemistry values: In the male rats, the groups receiving MPE at the doses of 500 and 1000 mg/kg/day had significantly higher ALT than the control group. AST and BUN in the highest dose and satellite group were significantly higher than those in the control group. Cholesterol of the group receiving MPE at the doses of 500 and 1000 mg/kg/day and of the satellite group showed significantly higher level than that of the control group. Total protein, uric acid and glucose in the highest dose group were significantly lower than those in the control group (Table 5). In the female, the highest dose group had significantly higher AST and total bilirubin than the control, whereas the glucose level was significantly lower than that in the control

Table 3 Hematological values of male rats receiving MPE for 6 months

Parameters	Dose of MPE administered (mg/kg/day)					
	Control	10	100	500	1000	1000-S
	n =15	n =15	n =15	n =15	n =15	n =15
Hematocrit (%)	32.35 ± 3.10	33.15 ± 2.57	31.35 ± 1.65	32.56 ± 2.59	31.66 ± 1.56	32.17 ± 1.44
RBC (×10^6cells/mm^3)	9.17 ± 0.75	9.33 ± 0.76	8.77 ± 0.43	9.08 ± 0.70	8.93 ± 0.58	8.87 ± 0.41
Hemoglobin (g/dl)	16.49 ± 1.05	16.78 ± 1.18	15.93 ± 0.42	16.58 ± 1.31	16.18 ± 0.98	16.34 ± 0.58
MCV (µm^3/red cell)	35.26 ± 1.46	35.57 ± 1.12	35.78 ± 1.19	35.85 ± 1.00	35.51 ± 1.02	36.31 ± 1.20
MCH (pg/red cell)	18.02 ± 0.78	18.02 ± 0.61	18.20 ± 0.72	18.28 ± 0.76	18.13 ± 0.57	18.44 ± 0.46
MCHC (g/dl RBC)	51.21 ± 3.32	50.68 ± 1.26	50.87 ± 1.57	50.96 ± 1.26	51.06 ± 1.27	50.79 ± 0.78
WBC (×10^3cells/mm^3)	2.47 ± 0.65	2.80 ± 0.58	2.52 ± 0.66	2.64 ± 0.73	2.59 ± 0.48	3.08 ± 0.73
Neutrophil (%)	27.50 ± 4.95	28.64 ± 6.36	29.56 ± 6.24	30.46 ± 9.39	38.63 ± 9.11*	29.80 ± 6.95*
Eosinophil (%)	2.04 ± 0.52	1.69 ± 0.49	1.94 ± 0.52	1.30 ± 0.43*	0.91 ± 0.34*	1.40 ± 0.46*
Lymphocyte (%)	65.20 ± 8.66	64.86 ± 7.27	64.10 ± 6.05	65.53 ± 10.22	56.73 ± 9.98	63.37 ± 8.05
Monocyte (%)	4.26 ± 3.52	3.95 ± 3.18	3.60 ± 3.20	2.15 ± 2.03	3.06 ± 2.43	3.25 ± 3.28
Basophil (%)	1.08 ± 1.06	0.86 ± 0.69	0.80 ± 0.51	0.55 ± 0.42	0.68 ± 0.50	1.30 ± 0.91
Platelet (×10^3cells/mm^3)	946.60 ± 126.81	918.37 ± 145.90	872.30 ± 112.18	962.40 ± 97.25	948.79 ± 121.76	895.77 ± 91.22

The values are expressed as mean±SD, 1000-S: the satellite group * significantly different from control group (p<0.05)

Table 4 Hematological values of female rats receiving MPE for 6 months

Parameters	Dose of MPE administered (mg/kg/day)					
	Control	10	100	500	1000	1000-S
	n =15	n =15	n =15	n =15	n =15	n =15
Hematocrit (%)	31.09 ± 2.46	31.35 ± 1.28	30.65 ± 1.92	30.40 ± 1.26	31.28 ± 2.18	32.03 ± 2.08
RBC (x 10^6cells/mm^3)	8.34 ± 0.76	8.26 ± 0.31	8.24 ± 0.46	7.98 ± 0.22	8.48 ± 0.59	8.45 ± 0.60
Hemoglobin (g/dl)	16.04 ± 1.17	16.16 ± 0.68	15.90 ± 0.99	15.71 ± 0.49	16.24 ± 0.91	16.42 ± 1.06
MCV (μm^3/red cell)	37.32 ± 1.01	37.98 ± 0.91	37.21 ± 1.09	38.11 ± 1.48	36.92 ± 0.65	37.93 ± 1.12
MCH (pg/red cell)	19.26 ± 0.80	19.57 ± 0.68	19.30 ± 0.68	19.70 ± 0.74	19.18 ± 0.57	19.45 ± 0.53
MCHC (g/dl RBC)	51.65 ± 1.35	51.54 ± 1.36	51.88 ± 0.99	51.73 ± 1.29	51.97 ± 1.31	51.27 ± 0.63
WBC (xx10^3cells/mm^3)	1.13 ± 0.31	1.22 ± 0.52	1.21 ± 0.38	1.43 ± 0.56	1.53 ± 0.49	1.74 ± 0.37*
Neutrophil (%)	31.11 ± 5.97	30.19 ± 6.92	32.24 ± 7.77	30.44 ± 7.17	29.75 ± 5.27	23.80 ± 6.09
Eosinophil (%)	2.02 ± 0.72	1.80 ± 0.93	1.58 ± 0.74	1.13 ± 0.87*	1.06 ± 0.60*	1.49 ± 0.66*
Lymphocyte (%)	62.23 ± 7.08	65.12 ± 7.10	61.79 ± 8.19	65.20 ± 7.80	66.04 ± 5.91	72.01 ± 5.99
Monocyte (%)	3.85 ± 2.94	2.33 ± 1.62	3.71 ± 2.34	2.59 ± 2.28	2.46 ± 1.90	1.85 ± 2.17
Basophil (%)	0.79 ± 0.64	0.55 ± 0.27	0.67 ± 0.35	0.65 ± 0.50	0.68 ± 0.30	0.84 ± 0.66
Platelet (x10^3cells/mm^3)	822.68 ± 92.86	763.73 ± 56.44	798.37 ± 64.25	824.29 ± 92.97	878.73 ± 94.02	878.93 ± 73.24

The values are expressed as mean±SD, 1000-S: the satellite group
* significantly different from control group (p<0.05)

Table 5 Biochemical values of male rats receiving MPE for 6 months

Parameters	Dose of MPE administered (mg/kg/day)					
	Control	10	100	500	1000	1000-S
	n =15	n =15	n =15	n =15	n =15	n =15
ALP (U/L)	54.40 ±12.23	51.00 ± 5.50	52.13 ± 10.74	48.93 ± 5.55	47.43 ± 7.27	52.93 ± 9.00
ALT (U/L)	44.13 ± 16.04	47.47 ± 21.83	36.20 ± 7.33	79.80 ± 21.73*	94.21 ± 40.31*	79.47 ± 45.68
AST (U/L)	97.13 ± 24.80	105.13 ± 34.59	91.73 ± 11.06	117.27 ± 36.84	137.00 ± 31.65*	132.00 ± 21.26*
Total protein (g/dl)	7.19 ± 0.19	7.05 ± 0.22	7.11 ± 0.29	6.95 ± 0.26	6.78 ± 0.33*	7.13 ± 0.27
Albumin (g/dl)	4.42 ± 0.13	4.41 ± 0.19	4.48 ± 0.15	4.50 ± 0.19	4.54 ± 0.25	4.49 ± 0.15
Total bilirubin(mg/dl)	0.08 ± 0.03	0.08 ± 0.03	0.09 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.10 ± 0.03
BUN (mg/dl)	18.97 ± 1.53	20.35 ± 2.43	18.59 ± 3.49	21.13 ± 2.31	23.38 ± 5.39*	25.07 ± 3.99*
Creatinine (mg/dl)	0.58 ± 0.04	0.58 ± 0.04	0.57 ± 0.05	0.52 ± 0.06	0.60 ± 0.16	0.60 ± 0.09
Glucose (mg/dl)	263.40 ± 57.23	250.48 ± 57.50	271.64 ± 70.96	208.45 ± 39.83	156.85 ± 46.71*	211.77 ± 40.50
Uric acid (mg/dl)	5.09 ± 1.32	5.24 ± 1.21	4.95 ± 1.65	4.39 ± 1.11	3.35 ± 1.13*	3.77 ± 1.30
Triglyceride(mg/dl)	81.78 ± 26.27	87.83 ± 45.58	92.42 ± 34.23	70.83 ± 22.72	64.21 ± 14.35	58.70 ± 11.23
Cholesterol (mg/dl)	84.90 ± 13.65	75.82 ± 12.91	79.34 ± 16.13	70.28 ± 13.99*	70.05 ± 9.48*	62.92 ± 8.89*
Sodium	145.07 ± 2.28	145.07 ± 2.49	145.60 ± 2.41	146.00 ± 2.51	146.64 ± 3.15	144.80 ± 1.78
Potassium	7.18 ± 0.65	7.24 z 0.75	6.67 ± 0.60	6.87 z 0.64	6.89 ± 0.93	7.61 ± 0.89
Chloride	102.87 ± 1.36	101.93 z 1.44	102.53 ± 2.07	102.33 ± 1.95	103.64 ± 2.37	104.40 ± 1.99

The values are expressed as mean±SD, 1000-S: the satellite group
* significantly different from control group (p<0.05)

Table 6 Biochemical values of female rats receiving MPE for 6 months

Parameters	Dose of MPE administered (mg/kg BW/day)					
	Control	10	100	500	1000	1000-S
	n =15	n =15	n =15	n =15	n =15	n =15
ALP (U/L)	22.27 ± 7.01	24.67 ± 5.92	23.87 ± 3.83	24.57 ± 4.36	24.87 ± 5.08	24.80 ± 4.25
ALT (U/L)	33.53 ± 8.68	31.80 ± 7.39	31.07 ± 6.35	40.14 ± 10.03	43.60 ± 15.99	24.87 ± 5.24
AST (U/L)	104.27 ± 16.02	114.93 ± 14.02	123.60 ± 23.72	119.29 ± 15.45	126.00 ± 19.61*	84.93 ± 7.58*
Total protein (g/dl)	7.15 ± 0.34	7.17 ± 0.23	7.13 ± 0.28	7.12 ± 0.30	7.16 ± 0.36	7.33 ± 0.34
Albumin (g/dl)	4.89 ± 0.25	4.92 ± 0.12	4.83 ± 0.23	4.76 ± 0.24	4.83 ± 0.27	4.87 ± 0.21
Total Bilirubin(mg/dl)	0.10 ± 0.03	0.12 ± 0.03	0.12 ± 0.04	0.14 ± 0.05	0.16 ± 0.04*	0.13 ± 0.02
BUN (mg/dl)	21.78 ± 2.61	24.66 ± 4.52	23.73 ± 5.02	28.21 ± 4.85*	30.96 ± 8.69*	30.07 ± 7.27*
Creatinine (mg/dl)	0.51 ± 0.05	0.58 ± 0.11	0.56 ± 0.06	0.62 ± 0.08*	0.68 ± 0.15*	0.64 ± 0.06*
Glucose (mg/dl)	107.61 ± 21.50	94.00 ± 16.53	99.00 ± 19.74	93.25 ± 20.21	77.87 ± 16.58*	100.85 ± 20.51
Uric acid (mg/dl)	2.43 ± 0.63	2.18 ± 0.39	2.07 ± 0.55	2.09 ± 0.67	2.45 ± 0.94	2.65 ± 0.75
Triglyceride (mg/dl)	41.33 ± 6.82	42.56 ± 6.20	38.96 ± 7.38	35.82 ± 7.58	38.40 ± 9.36	44.85 ± 7.67
Cholesterol (mg/dl)	67.55 ± 12.94	61.86 ± 18.23	63.46 ± 13.82	66.15 ± 13.45	62.35 ± 13.01	64.13 ± 14.15
Sodium	145.20 ± 2.51	145.67 ± 2.06	146.40 ± 2.23	146.71 ± 2.70	147.53 ± 1.96	145.93 ± 1.39
Potassium	6.29 ± 0.96	6.00 ± 0.94	5.42 ± 1.17	5.32 ± 1.38	5.65 ± 1.43	7.15 ± 1.60
Chloride	106.13 ± 2.61	105.13 ± 1.73	105.60 ± 1.59	105.43 ± 2.03	104.07 ± 2.22	107.33 ± 1.88

The values are expressed as mean±SD, 1000-S: the satellite group
* significantly different from control group (p<0.05)

group. Both BUN and creatinine in the group receiving MPE at dose of 500 mg/kg/day onward and those in the satellite group were significantly higher than those in the control group (Table 6).

Effects of MPE on histopathological alterations: At autopsy, there was no remarkable macroscopic lesions in any organs of both MPE-treated and control groups. Histopathology of visceral organs revealed that the highest dose male and female groups had significantly lower incidence of bronchiole-associated lymphoid tissue proliferation in the lung than their corresponding control groups. The satellite group of both sexes had significantly higher incidence of centrilobular hydropic degeneration in the liver tissue than their corresponding control group. Histopathological findings of the heart, kidney and intestine in all MPE-treated groups did not differ from those in the control group (Table 7 and 8). In addition, there was no remarkable lesion in other examined organs between the MPE-treated and control group.

Table 7 Histopathological results of male and female rats receiving MPE for 6 months

Organs	Microscopic findings	Male rats						Female rats					
		Dose of MPE administered (mg/kg/day)						Dose of MPEadministered (mg/kg/day)					
		Control	10	100	500	1000	1000-S	Control	10	100	500	1000	1000-S
Lung	BALT proliferation	8/15	9/15	7/15	6/15	2/15*	6/15	9/15	5/15	5/15	4/15	1/15*	6/15
Heart	Myocardiosis	1/15	1/15	0/15	0/15	0/15	1/15	NRL	NRL	NRL	NRL	NRL	NRL
Liver	Centrilobular hydropic degeneration	2/15	0/15	0/15	0/15	6/15	9/15*	0/15	0/15	0/15	0/15	2/15	8/15*
Kidney	Dilated tubule	0/15	0/15	0/15	0/15	1/15	4/15*	NRL	NRL	NRL	NRL	NRL	NRL
	Hydronephrosis	NRL	NRL	NRL	NRL	NRL	NRL	0/15	0/15	1/15	4/15*	0/15	0/15
Small intestine	GALT proliferation in submucosa	1/15	3/15	2/15	3/15	1/15	1/15	1/15	3/15	1/15	2/15	1/15	1/15
Large intestine	GALT proliferation in submucosa	1/15	2/15	1/15	5/15	2/15	2/15	2/15	1/15	3/15	7/15	1/15	1/15

The results were expressed as the number of rats with pathological findings per total number of rats treated, 1000-S: the satellite group

* significantly different from control group (p<0.05)

(NRL: No remarkable lesions, BALT: Bronchiole-associated lymphoid tissue, GALT: Gut-associated lymphoid tissue)

Discussion

In this study, an administration of MPE at any tested doses did not cause any overt toxic signs and mortality in the rats. The measurement of body weight indicated that MPE may depress the growth of the animals and the male rats were more susceptible to this effect than the female rats. Our result was different from a previous study by Towatana et al. (2010) saying that the oral administration of the 50% ethanolic extract in Wistar rats for three months did not affect the body weight at any time-points. This discrepancy may be caused by the difference in chemical constituents and their contents between the 95% and 50% ethanolic extract. Peaslee and Einhellig (1973) demonstrated that mice fed with diet containing tannic acid had retarded growth. In addition, the weanling rats receiving high tannin varieties of sorghum had significantly lower growth than those treated with low tannin varieties (Jambunathan and Mertz, 1973). Therefore, the result of poorer body weight in the male group treated with 500 mg/kg MPE and that in the highest dose of both sexes might partially be due to the effects of tannins. The significantly less food intake were observed at only three time-points in the male rats of highest dose group and only one time-point in the female receiving this dose. However, measurement of the weekly food intake at any other time-points in both groups did not show any discrepancies. Thus, it could not be stated that MPE suppressed the food intake of the animals.

Almost all of the organs of the male rats of the highest dose and satellite groups as well as several organs of the male rats treated with 500 mg/kg/day MPE revealed higher relative weights. These findings may be caused by the lower body weight. As histopathological results of such organs did not show any associated abnormalities. In addition, their actual weights did not reveal any changes when compared to the control group. Similar reasons may account for the increased relative weight of some organ of the female rats of the highest dose and satellite groups.

Hematological analysis of blood samples from the female rats revealed no significant difference in the total WBC number between the highest dose and their control group. Thus, the increase of such parameter in only the satellite female group could due to biological variations among rats rather than the results of the extract. A decrease of eosinophils in both male and female groups receiving MPE at the doses of 500 and 1000 mg/kg/day as well as that in the satellite groups was likely due to the extract; however, this alteration was within normal range (Gad, 1992) and revealed no clinical significance. Neutrophil counts of the male rats receiving the highest dose was significantly higher and seemed to be dose-related. While Jejun et al. (2008) found that the same dose of 95% ethanolic extract of mangosteen did not cause any significant difference in hematological parameters including neutrophil in the male Sprague-Dawley rats. This discrepancies may be due to the difference in rat strains and duration of extract treatment. However, in this study, the alteration of neutrophils was slightly higher than the normal range (Gad, 1992) and the withdrawal of treatment could lead to reversibility of this effect. Clinical chemistry examination revealed the increase of ALT levels in the male rats receiving MPE at the

doses of 500 and 1000 mg/kg/day and also that of AST in the male and female rats receiving highest dose, which may be caused by MPE. Pramyothin et al. (2003) demonstrated that xanthones, the major compound isolated from mangosteen pericarp, caused the increase of both transaminases enzymes in isolated rat hepatocytes, which indicated the hepatoxic effect. In addition, mangostin has been shown to induce the significant increase of AST and ALT in rats treated when given by intraperitoneal or oral administration (Sornprasit et al., 1987). The decrease of total protein in the male rats receiving highest dose was slightly lower than the normal range (Pimainog et al., 2003) and this change could return to normal after the extract withdrawal. Possible causes of this finding may be decreased protein synthesis caused by hepatic insufficiency and/or increased protein catabolism (Stockham and Scott, 2002). Marzo et al. (2002) reported that chicken fed with tannic acid added diet exhibited a marked increase in the activities of liver cathepsin A and D which suggested the increase in protein catabolism. Thus, the tannin in MPE, at least in part, account for this finding. The increase of total bilirubin in the female rats receiving the highest dose was within normal range (0.00-0.55 mg/dl) as reported by Gad (1992).

The decrease of glucose levels in the males and females receiving highest dose might contribute to the decreased gluconeogenesis in the liver, according to the hepatic insufficiency as shown by the increased hepatic enzyme activities (Stockham and Scott, 2002). The decrease of uric acid level in the male rats tended to be dose-related, however that in the highest dose group was within normal range (Gad, 1992). The decrease of cholesterol levels in the male rats receiving the extract at 500 mg/kg onward as well as the male satellite group might be due to the decreased production in the impaired hepatocytes (Stockham and Scott, 2002); nevertheless this alteration was within the reference values of the male rats (Pimainog et al., 2003). The increase of BUN and creatinine in the female rats treated with 500 and 1000 mg/kg/day as well as the increase of BUN in the male rats of the highest dose group were higher than the reference interval of male and female Wistar rats as reported by Pimainog et al. (2003). These alterations suggested that MPE might affect the kidney function and this effect was also observed in both sexes after the extract withdrawal.

Histopathological examination of the lungs from the male and female rats of the highest dose group revealed a decreased incidence of bronchiole-associated lymphoid tissue proliferation. This finding might be due to the anti-inflammatory effect of the extract. Nakatani et al. (2002) demonstrated that γ-mangostin, a xanthone compound in the mangosteen fruit hull, inhibited the syntheses of prostaglandin E2 and enzymes cyclooxygenase I and II. A significantly higher incidence of centrilobular hydropic degeneration of the liver of the male rats of the satellite group, together with the increase AST levels may be indicative of hepatotoxic effects of the extract. While the female rats of the satellite group had an increase incidence of such histological alteration without any elevations of their AST or ALT enzymes. The reasons for this finding may be explained that the magnitude of the serum enzyme activity is not a reliable indicator regarding the type or degree of tissue injury (Lassen, 2004). Our results also suggested that the withdrawal of MPE treatment could not lead to reversibility of hepatotoxic effects within two weeks. Although the males of the satellite group had the higher incidence of the kidney tubular cyst, this alteration did not show any significant difference between the male rats of the highest dose and their control group. Furthermore, the female rats of the satellite and the highest dose group did not possess this alteration in their kidneys. Thus, this finding may not be concluded to be the results of MPE and needs to be further investigated. The increase of hydronephrosis incidence in the female rats receiving the extract at dose of 500 mg/kg/day could not be due to MPE since this was not dose dependent. Moreover, this change is considered incidental and thought to be congenital and inherited in many strains of rats (King and Russel, 2006). Other histopathological findings in the treatment groups did not show any dose dependency or any significant difference; therefore it could not be due to MPE.

In conclusion, the six-month oral administration of MPE in Wistar rats at the doses of 10, 100, 500 and 1000 mg/kg/day revealed that MPE did not produce any overt pharmacotoxic signs and abnormalities in hematological values. However, MPE at dose of 500 mg/kg/day onward affected the body weight and produced the increase in ALT, BUN and creatinine in the tested animals. The highest dose MPE caused the significant increase in AST. In addition, the finding of hepatocellular degeneration after the highest dose withdrawal may be suggestive of the persistence in liver pathology caused by the highest dose extract. Hence, this study revealed that MPE at dose 500 mg/kg onward affected liver and kidney, and it could not be suggested that MPE are safe for the long term usage. Additional assessment of the appropriate dose range including further studies on hepatotoxicity of various chemical components in MPE are suggested to be investigated, which may be useful for the safe assessment and for the health product development from mangosteen pericarp extract.

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