

## Acute and Subchronic Toxicity of Mulberry Fruits

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### ABSTRACT

At present, the application of phytomedicine worldwide is substantial increase. The data of the acute and subchronic toxicity studies on medicinal plants or fruits and vegetables used for medicinal purposes should be obtained in order to increase the confidence in its safety to human, particularly for the application as functional food. Despite the widely consumption of mulberry (*Morus alba* L, Moraceae family) fruits both as fruit and as medicine, no systematic evaluation of the toxicity of mulberry fruits is available until now. This study aimed to determine the acute and subchronic oral toxicities of mulberry fruits in male and female Wistar rats. A single acute mulberry fruits extract dose of 2000 mg kg<sup>-1</sup> dissolved in distilled water was administered by oral gavage for acute toxicity. Subchronic doses of 2, 10 and 500 mg kg<sup>-1</sup> day<sup>-1</sup> were also administered the same way for 90 days. The major toxicological endpoints examined included animal body weight, water and food intake, selected tissue weights and histopathological examinations. In addition, we also examined hematological and clinical chemistry values. The results showed no abnormalities in treated groups as compared to the controls. Although significantly different BUN and glucose were also observed, all of the values were within normal limits. Neither gross abnormalities nor histopathological changes were observed. The results suggest that mulberry fruits do not produce acute or subchronic toxicity in either female or male rats. Therefore mulberry fruits have the potential to be use as functional food. However, a chronic toxicity study should be further carried out to assure the safety for repetitive administration of this fruit before the application of functional food.

**Keywords:** Mulberry Fruits, Acute Toxicity, Subchronic Toxicity

### 1. INTRODUCTION

At present, there has been a substantial increase in the use of Complimentary Alternative Medicine. Due to the widespread use of Traditional and Complementary Alternative Medicine and the tremendous expansion of international markets for herbal products, it is all the more important to ensure that the health care provided by Traditional and Complementary Alternative Medicine is safe and reliable (Zhang, 2001). Among various types of Complimentary Alternative Medicine, phytomedicine is the most widely used.

Since ancient times, plants have been valued for their medicinal properties and many cultures still rely on plants as their major source of medicine. Numerous fruits and vegetables have been used as food and as one

of the main source of drug discovery and development. However, only a few of them have been scientifically investigated, especially about their toxic aspects.

Mulberry (*Morus alba* L.) or Mohn in Thai, a plant in family of Moraceae, is widely cultivated in the North and North East of Thailand. Mulberry fruit is widely regarded as a nutritious food. It can be eaten freshly or widely used in the production of wine, fruit juice, jam and canned food (Ning *et al.*, 2005). Mulberry fruit is not only used as fruit but also as medicine. According to the traditional folklore, mulberry fruit is used to protect against liver and kidney damage, strengthen the joints, improve eyesight and have anti-aging effects (Li, 1982). It is also used for the treatment of sore throat, fever, hypertension and anemia (Ma, 2002; Gong and Zhu,

2008). In addition, recent findings showed that mulberry fruits could protect against brain damage in various conditions including Parkinson's disease (Kim *et al.*, 2010) and cerebral ischemia (Kang *et al.*, 2006). Moreover, mulberry fruit extract also decreased  $\beta$ -amyloid protein and improved learning impairment in Senescence-Accelerated Mice (SAMP) (Shih *et al.*, 2010).

Although the safety of mulberry fruits can be assumed from their very long history of consumption in Asian countries, systematic evaluation of the toxicity of mulberry fruits has been lacking. Therefore, the purpose of this study was to investigate acute and subchronic oral toxicities of mulberry fruits.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of Mulberry Fruits Freeze-Dried Powder

All mulberry fruits (*Morus alba* var Chiangmai) used in this study is prepared and provided by The Institute of Queen Sirikit Seri Culture, Thailand. Mulberry fruits were collected from the Queen Sirikit Seri Culture Center Udon Thani. All berries were picked at the commercially ripen stage and selected according to uniformity color. Then, the fruits were dried at 70°C for 4 days and grounded to powder. In addition, the mulberry fruit powders were also kept in dark air tight bottle at -20°C. The mulberry fruit powder used in this study contained phenolic compounds at concentration of 519±0.004 mg GAE/g fresh matter of fruit. It also contained anthocyanin at concentration of 667.95±0.07 mg of Cyanidin-3-Glucoside Equivalents (CGE) per 100 g of fresh weight.

### 2.2. Animal

Adult male Wistar rats, 8 weeks old, were used as experiment animals. They were obtained from National Animal Center, Salaya, Nakornpatom. The weights of the animals on the first day of experiment were 180-220 grams. All animals were given access to food and water ad libitum. The experiments were performed to minimize animal suffering and the experimental protocols will be approved by the Institutional Animal Care and Use Committee Khon Kaen University, Thailand.

### 2.3. Acute Toxicity Test

Acute toxicity test was performed according to the World Health Organization (WHO) guideline (WHO, 1986) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (OECD, 2001). Fifteen rats per sex were administered a single oral dose of 2,000 mg kg<sup>-1</sup> body weight while the control group received water vehicle.

Body weight, signs of toxicity and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for next 14 days. On the 15th day, all rats were kept fasted for 16-18 h and then sacrificed for necropsy examination. The internal organs were excised and weighed. The gross pathological observations of the tissues were performed.

### 2.4. Subchronic Toxicity Test

According to WHO guideline (WHO, 1986) and the OECD (OECD, 1981), rats were divided into 5 groups of 30 animals (15 male and 15 female). According to the previous study, we have found that the effective doses 2-10 mg kg<sup>-1</sup> BW of powdered fruits significantly exerted the neuroprotection and cognitive enhancer effects. In the present study, the doses of mulberry fruits were 2, 10 and 500 mg kg<sup>-1</sup> day<sup>-1</sup>, which are cover the effective doses and the equivalent 50-250 times of effective dose (2-10 mg kg<sup>-1</sup>). The mulberry fruits powder were dissolved in distilled water and orally given to each group of rats daily for 90 days, while the control group received the water vehicle. Toxic manifestations such as signs of toxicity, mortality and the body weight changes were monitored daily. At the end of the study, all animals were fasted for 16-18 h and then anesthetized with intraperitoneal injection of pentobarbital sodium at a dose of 50 mg kg<sup>-1</sup> on day 91st. Blood samples for hematological and blood chemical analyses were taken from abdominal aorta. All rats were sacrificed after the blood collection. The internal organs and some tissues were weighed to determine relative organs weights and observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

### 2.5. Statistical Analysis

Results were expressed as mean ± Standard Error of Mean (SEM). Statistical significance was determined by one-way Analysis of Variance (ANOVA) and post hoc Least-Significant Difference (LSD) test. The data obtained from acute toxicity studies were analyzed using Student's paired t-test. P values less than 0.05 were considered significant.

## 3. RESULTS

### 3.1. Acute Toxicity

The powder of mulberry fruits at a single dose of 2000 mg kg<sup>-1</sup> was orally given to rats. Neither sign of toxicity nor death of rats was observed during the 14 days of the experimental period. Toxicity evaluation was further carried out by observing both body weight gain and internal organ weight. **Table 1** showed that no significant changes of the parameters mentioned earlier were observed. Furthermore, gross examinations of the internal

organs of treated rats revealed no pathological abnormality as compared with those of the control (data not shown).

### 3.2. Subchronic Toxicity

Neither changes in animal behaviors nor toxic signs were detected in the treatment rats. The body weight and internal organ weight of both male and female rats after repetitive administration of mulberry fruits at doses of 2, 10 and 500 mg kg<sup>-1</sup> BW were shown in **Table 2 and 3**. Our data clearly demonstrated that no significant changes in the parameters just mentioned were observed.

All hematological values of male and female tested in this study were shown in **Table 4 and 5**. There were no treatment-related changes in all hematological parameters tested in this study. The blood chemistry values of male and female rats subjected to mulberry fruits treatment at all doses used in this study were demonstrated in **Table 6 and 7**. **Table 6** showed that the Blood Urea Nitrogen

(BUN) of male rats significantly decreased ( $p < 0.05$  compared to control). However, these values were still in the normal range (Johnson-Delaney and Harrison, 1996). No other changes were observed in this study. The BUN value of female rats subjected to mulberry fruits treatment at dose of 500 mg kg<sup>-1</sup> BW also showed the significant reduction of BUN as shown in **Table 7**. Again, this change was still in the normal range (Johnson-Delaney and Harrison, 1996). Moreover, all doses of mulberry fruits could produce significant elevation of glucose levels in female rats ( $p < 0.05$  all; compared to control). The changes of blood sugar levels were also in the normal range (Johnson-Delaney and Harrison, 1996). No other significant clinical chemistry values were observed in both male and female rats. In addition, the results of the histopathological assessment showed no significant histopathological change in the internal, especially vital organs.

**Table 1.** The effect of mulberry fruits on body weight gain and internal organ weight.

Organs	Dose of <i>M. alba</i> (g/kg BW/day)			
	Male- Control	Male 2g/kgBW	Female-Control	Female 2g/kgBW
Final body weight	281.80±4.09	282.33±9.54	255±3.72	243±3.63
Brain	7.10±0.12	6.76±0.24	7.85±0.18	7.73±0.16
Lung	8.20±0.35	10.30±0.72	7.76±0.32	7.82±0.51
Heart	4.25±0.18	4.45±0.18	3.42±0.08	3.46±0.08
Liver	34.67±1.55	38.62±1.51	33.17±0.74	35.92±1.34
Right Kidney	4.03±0.12	4.08±0.14	3.45±0.13	3.33±0.08
Left Kidney	4.16±0.15	4.22±0.15	3.31±0.07	3.46±0.08
Spleen	3.80±0.27	4.05±0.18	2.74±0.17	2.92±0.09
Pancreas	6.82±0.38	6.27±0.55	5.83±0.23	5.77±0.37
GI Tract	79.07±1.91	84.83±3.33	96.41±1.32	94.94±4.24
Right Testis/Ovary	7.11±0.16	7.08±0.25	2.03±0.16	2.15±0.12
Left Testis/Ovary	7.33±0.24	7.08±0.3	2.28±0.25	2.21±0.18
Adrenal gl.	0.38±0.04	0.40±0.05	0.40±0.06	0.46±0.04
Right Salivary gland	0.61±0.04	0.64±0.02	0.52±0.04	0.55±0.04
Left Salivary gland	0.71±0.06	0.66±0.05	0.54±0.04	0.53±0.03
Thymus	1.77±0.05	1.50±0.16	1.52±0.13	1.48±0.09

\* significantly different from control group ( $p < 0.05$ ) The values are expressed as mean ± S.E.M.)

**Table 2.** Body weight (g) and relative organ weight (g/kg) of male rats receiving *M. alba* for 3 months

Organs	Dose of <i>M. alba</i> (mg/kg BW/day)			
	Control	21	0	500
Initial body weight	206.00±4	234.000±12	220.000±2	234.000±13
Final body weight	398.10±8	378.000±8	435.000±6*	420.000±7*
Brain	5.02±0.06	5.110±0.13	4.810±0.10	4.850±0.11
Lung	5.80±0.23	6.690±0.66	6.570±0.37	6.490±0.67
Heart	3.02±0.16	3.280±0.08	3.050±0.05	3.050±0.06
Liver	24.51±1.00	26.860±0.62	24.720±0.41	25.310±0.65
Right Kidney	2.85±0.09	3.030±0.10	2.910±0.07	2.920±0.09
Left Kidney	2.95±0.12	3.130±0.10	2.780±0.10	3.000±0.09
Spleen	2.69±0.43	3.000±0.12	2.280±0.14	2.460±0.09
Pancreas	4.83±0.28	4.640±0.41	4.310±0.35	4.660±0.35
GI Tract	55.90±1.02	56.670±2.03	53.980±1.24	56.250±1.18
Right Testis	5.03±0.1	5.250±0.17	5.020±0.11	4.850±0.15
Left Testis	5.19±0.15	5.240±0.19	5.010±0.07	4.540±0.27
Adrenal gl.	0.27±0.03	0.300±0.03	0.341±0.026	0.290±0.02
Right Salivary gland	0.44±0.05	0.482±0.018	0.421±0.027	0.420±0.024
Left Salivary gland	0.51±0.05	0.485±0.035	0.451±0.036	0.502±0.049
Thymus	1.25±0.04	1.240±0.11	1.230±0.03	1.260±0.07

\* significantly different from control group ( $p < 0.05$ ) The values are expressed as mean ± S.E.M.

**Table 3.** Body weight (g) and relative organ weight (g/kg) of female rats receiving *M. alba* for 3 months

Organs	Dose of <i>M. alba</i> (mg/kg BW/day)			
	Control	2	10	500
Initial body weight	219.000±3	231.000±3*	219.000±2	224.000±6
Final body weight	255.000±4	251.000±3	250.000±2	255.000±2
Brain	7.860±0.18	7.460±0.13	7.750±0.09	7.870±0.15
Lung	7.770±0.32	8.400±0.52	7.610±0.51	8.030±0.55
Heart	3.420±0.09	3.340±0.07	3.350±0.07	3.450±0.07
Liver	33.180±0.75	34.980±0.93	34.740±1.07	32.870±1.12
Right Kidney	3.460±0.13	3.230±0.08	3.520±0.11	3.370±0.12
Left Kidney	3.320±0.08	3.340±0.07	3.640±0.18	3.250±0.09
Spleen	2.740±0.17	2.820±0.09	2.900±0.16	2.740±0.12
Pancreas	5.840±0.22	5.590±0.37	6.170±0.37	6.070±0.45
GI Tract	96.420±1.32	96.080±3.25	94.760±4.57	90.140±3.71
Right Ovary	2.040±0.17	2.080±0.12	2.070±0.13	1.830±0.20
Left Ovary	2.290±0.25	2.130±0.18	2.420±0.29	1.810±0.18
Adrenal gl.	0.406±0.061	0.448±0.041	0.409±0.041	0.432±0.036
Right Salivary gland	0.522±0.042	0.538±0.043	0.564±0.038	0.521±0.029
Left Salivary gland	0.541±0.045	0.512±0.037	0.529±0.034	0.580±0.063
Thymus	1.520±0.13	1.430±0.09	1.49 ± 0.07	1.540±0.11

\* significantly different from control group ( $p < 0.05$ ) The values are expressed as mean  $\pm$  S.E.M.)

**Table 4.** The effect of mulberry fruits on all hematological values of male for 3 months

Parameters	Dose of <i>M. alba</i> (mg/kg BW/day)			
	Control	2	10	500
Hematocrit (%)	48.30±1.61	47.11±1.55	44.040±4.48	46.210±0.64
RBC ( $\times 10^6$ cells/mm <sup>3</sup> )	7.91±0.30	7.53±0.53	7.680±0.74	8.150±0.10
Hemoglobin (g/dl)	16.53±0.23	14.89±0.70	15.160±1.47	16.050±0.18
MCV ( $\mu\text{m}^3$ /red cell)	61.39±1.97	58.43±0.73	52.280±5.17	56.700±0.43
MCH (pg/red cell)	21.15±0.78	18.53±0.71	18.110±1.77	19.720±0.17
MCHC (g/dl RBC)	34.54±1.18	31.74±1.22	31.560±3.13	34.770±0.22
WBC ( $\times 10^3$ cells/mm <sup>3</sup> )	4.39±0.39	3.90±0.25	3.880±0.55	3.650±0.35
Neutrophil (%)	14.88±2.01	19.94±1.47	16.780±2.16	19.037±0.78
Lymphocyte (%)	73.39±3.87	66.32±1.79	61.840±6.26	67.970±1.68
Monocyte (%)	8.20±1.81	8.55±0.87	8.024±1.01	8.180±0.94
Eosinophil (%)	3.19±0.76	3.74±0.61	3.791±0.56	4.160±0.58
Basophil (%)	0.34±0.02	0.33±0.04	0.360±0.07	0.320±0.02
Platelet ( $\times 10^3$ cells/mm <sup>3</sup> )	593.30±74.36	587.86±43.29	593.480±82.23	575.920±47.40

\* significantly different from control group ( $p < 0.05$ ) The values are expressed as mean  $\pm$  S.E.M.)

**Table 5.** The effect of mulberry fruits on all hematological values of female for 3 months

Parameters	Dose of <i>M. alba</i> (mg/kg BW/day)			
	Control	2	10	500
Hematocrit (%)	48.25±0.82	49.25±0.80	44.98±1.20	49.77±1.54
RBC ( $\times 10^6$ cells/mm <sup>3</sup> )	8.23±0.15	8.32±0.12	7.64±0.21	8.14±0.16
Hemoglobin (g/dl)	16.15±0.21	16.21±0.22	15.14±0.33	16.05±0.34
MCV ( $\mu\text{m}^3$ /red cell)	58.65±0.73	59.17±0.72	58.9±0.470	61.13±1.23
MCH (pg/red cell)	19.65±0.23	19.48±0.18	19.87±0.25	19.72±0.21
MCHC (g/dl RBC)	33.51±0.42	32.96±0.35	33.74±0.35	32.37±0.38
WBC ( $\times 10^3$ cells/mm <sup>3</sup> )	4.35±0.10	4.36±0.16	3.73±0.37	3.35±0.37
Neutrophil (%)	19.94±1.17	21.62±1.30	20.22±0.59	20.47±1.93
Lymphocyte (%)	71.84±1.71	71.88±1.61	70.81±1.10	66.60±4.09
Monocyte (%)	5.38±1.09	3.74±0.26	5.24±0.60	5.27±0.55
Eosinophil (%)	1.93±0.40	2.20±0.45	2.86±0.54	2.81±0.37
Basophil (%)	0.33±0.04	0.55±0.10	0.40±0.04	0.55±0.09
Platelet ( $\times 10^3$ cells/mm <sup>3</sup> )	685.5±32.04	675.26±50.42	695.33±51.31	682.07±51.72

\* significantly different from control group ( $p < 0.05$ ) The values are expressed as mean  $\pm$  S.E.M.)

**Table 6.** Biological values of male rats receiving *M. alba* for 3 months

Parameters	Dose of <i>M. alba</i> (mg/kg BW/day)			
	Control	2	10	500
AST (U/L)	186.80±9.60	192.60±8.46	221.27±9.77	226.67±13.35
Bilirubin (mg/dl)	2.54±0.21	1.99±0.16	2.59±0.11	3.36±0.21*
BUN (mg/ml)	25.20±3.09	16.93±0.50*	18.80±0.55*	17.47±1.05*
Glucose (mg/dl)	81.40±4.76	82.87±2.08	73.60±1.52	98.67±2.89*
Uric acid (mg/dl)	10.84±1.58	6.95±0.31*	8.35±0.36*	9.33±0.60
Triglyceride (mg/dl)	110.00±4.95	103.27±4.05	116.93±5.58	116.73±7.36
Cholesterol (mg/dl)	151.40±1.11	152.20±1.03	150.73±0.52	151.47±0.79

\* significantly different from control group ( $p < 0.05$ ) The values are expressed as mean  $\pm$  S.E.M.)

**Table 7.** Biological values of female rats receiving *M. alba* for 3 months

Parameters	Dose of <i>M. alba</i> (mg/kg BW/day)			
	Control	2	10	500
AST (U/L)	261.00±17.26	266.33±16.02	258.87±8.85	266.47±22.32
Bilirubin (mg/dl)	3.06±0.21	3.31±0.25	3.29±0.19	3.29±0.16
BUN (mg/ml)	26.80±1.32	26.60±0.87	26.20±0.81	22.80±0.93*
Glucose (mg/dl)	85.60±1.77	100.00±2.07*	97.13±1.85*	89.53±3.33
Uric acid (mg/dl)	7.64±0.48	8.17±0.43	7.59±0.45	8.16±0.56
Triglyceride (mg/dl)	123.00±11.42	113.00±6.80	129.33±9.14	126.70±9.26
Cholesterol (mg/dl)	151.00±0.80	150.40±0.21	150.67±0.41	151.53±0.96

\* significantly different from control group ( $p < 0.05$ ) The values are expressed as mean  $\pm$  S.E.M.)

#### 4. DISCUSSION

In acute toxicity study, mulberry fruits at a single dose of 2,000 mg kg<sup>-1</sup> did not show any toxicity signs (body weight, internal organ weight and general behaviors). The results suggest that mulberry fruits are practically not toxic after an acute exposure in rats.

Commonly, mulberry fruits could provide beneficial effects after the repetitive consumption over a long period. Unfortunately, little toxicity information is available regarding safety following repeated exposure. The effects of mulberry fruits exposure in subchronic regimen appeared to vary by sex. Generally, no changes in body weight, organ weights, hematological values and histopathological data were observed while a slight but significant reduction of BUN in both male and female were observed. The previous data which reported the effect of *Bryophyllum Pinnatum* (Lam.) on the hematological values showed non deference in Sprague-Dawley Rats (Raymond *et al.*, 2010). The reduction of this parameter might be due to the increase water concentration much more than the pathophysiology of liver because no data in this study indicated the liver damage. In addition, our data also demonstrated that the female rats showed trends to increase blood sugar level following mulberry administration much more than male. Based on the previous data which reported the difference in carbohydrate and fat metabolism between male and female (Gustavsson *et al.*, 2010) and high carbohydrate

especially fructose content in mulberry fruits, we did suggest that the increase blood sugar level in female rat might be associated with the lower carbohydrate metabolism in female rat. However, all changes observed in this study were still in normal range. Therefore, our data suggested that the No Adverse Effect Level (NOAEL) dose in both male and female were 500 mg kg<sup>-1</sup> BW.

#### 5. CONCLUSION

Mulberry fruits administered orally did not cause acute and subchronic toxicities in male or female rats. However, a chronic toxicity study should be further carried out to assure the safety for repetitive administration of this fruit before the application of functional food.

#### 6. ACKNOWLEDGEMENT

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