Chapter 4

Finding and Results

1. Materials and plant extracts of *T. chebula* and *T. bellerica*

The dried fruits of *T. chebula* and *T. bellerica* were extracted by separately boiled with water for 1 h and filtered. The water extract of each dried fruit is spray dried to remove trace of water. After that, the water extracts of *T. chebula* and *T. bellerica* were tested for quality controls. This includes physical appearance, percentage of loss on drying, total ash, acid insoluble ash, microbial test, aflatoxin test, heavy metal, and quantity of chemical compounds (percentage of tannins, total carbohydrate, uronic acid, and gallic acid), according to Thai Herbal Pharmacopoeia. The values of quality control and quantity of chemical compounds of the water extracts of *T. chebula* and *T. bellerica* were remained within the normal ranges, which are shown in table 4.1, 4.2, 4.3, and 4.4, respectively.

2. Acute toxicity study

In acute toxicity study, female and male rats fed with the water crude extracts from dried fruits of *T. chebula* and *T. bellerica* at a single dose of 5,000 mg/kg body weight were observed the signs of toxicity in the entire period of 14 days. Moreover, there were observing in body weight gain, internal organ weight, gross and pathological examinations of the internal organs.

Both female and male rats fed with the water extract from dried fruits of *T. chebula* at a dose of 5,000 mg/kg did not show any signs of toxicity thrughout the period of 14 days of observation. The body weight and internal organ weight are shown in table 4.5 and 4.6, respectively. Neither body weight nor internal organ weight of treated rats was significantly changed relative to that of the control group ($p<0.05$). The internal organs of treated rats such as lung, liver, kidney, spleen, adrenal grand, heart, pancreas, brain and sex organ showed no pathological abnormality relative to these organs of the control group (data not shown).

After the rats were orally given a single dose of the water crude extracts from dried fruits of *T. bellerica* at 5,000 mg/kg body weight. The body weight and organ weights of both male and female showed no significant changes when compared with the control group ($p< 0.05$) as shown in table 4.7 and 4.8, respectively. Furthermore, neither changes in animal behaviors nor toxic signs were detected in the treated rats. The pathological examination of the internal organs at a gross level revealed that there were no detectable abnormalities and no differences between the control and treated group. These results suggest that the water crude extracts from dried fruit of *T. chebula* and *T. bellerica* are practically not toxic after an acute exposure.

3. Chronic toxicity study

In the chronic toxicity study, rats were divided into 5 groups of 20 animals (10 female and 10 male). The rats were treated with the water extract from dried fruits of *T. chebula* and *T. bellerica* at all three doses (300, 600, and 1,200 mg/kg body weight /day) for 270 days, while the control group was given only the water vehicle.

3.1 Chronic toxicity study of *T. chebul*a

3.1.1 Effect on the general behavior

All of the rats fed with the water extract from dried fruits of *T. chebula* showed normal general behaviour, respiratory pattern, cardiovascular signs, motor activities, and reflexes. Besides, they exhibited normal changes in skin and fur (data not shown).

3.1.2 Effect on the body weight and organ weight

The body weight and body weight gain of both female and male rats treated at various concentrations (300, 600 and 1,200 mg/kg body weight) are presented in table 4.9. After the male rats were fed with the *T. chebula* extract, they showed a significant decrease in the body weight and body weight gain in comparison with the control group. As shown in table 4.10, the female rats treated with the extract at a dose of 600 mg/kg body weight/day had the brain, liver, spleen, and kidney

weight significantly lower than that of the control. Besides, the satellite female group showed a significant decease in the lung weight. In table 4.11, the male rats in the group treated with 300 mg/kg body weight/day showed a significant reduction of weights of both brain and liver, while the rats treated with 600 mg/kg body weight/day showed only a decrease in weight of liver. At the dose of 1,200 mg/kg body weight/day, a significant weight increase was also found in epidydimis when compared with the control ($p<0.05$).

3.1.3 Effect on the hematological values

Hematological parameters of female and male rats were examined as presented in table 4.12 and 4.13, respectively. The mean corpuscular volume (MCV) in female treated with 300 mg/kg body weight /day was significantly lower than that in the control group. In contrast, MCV in the satellite female group was significantly higher than that in the control group. In the male groups, the red blood cells in treated group with 300 mg/kg body weight/day were significantly higher than those in the control group. In the satellite male group, the red blood cells and hematocrit were significantly higher than that in the control group, whereas MCV of this group was significantly lower than those in the control group ($p<0.05$). However, the alteration of these values was minor and remained within the normal ranges.

The differential white blood cell count values of the female and male rats treated groups are shown in table 4.14 and 4.15, respectively. In the female, a significant increase in eosinophil was observed in the treated groups with 600 mg/kg body weight/day. Additionally, a significant decrease in lymphocyte and a significant increase in eosinophil were observed in the treated group with 1,200 mg/kg body weight/day as compared with the control values. Furthermore, a significant decrease in lymphocyte and a significant increase in neutrophil and monocyte were observed in the satellite group. In the male rats treated with 600, 1,200 mg/kg body weight/day and the satellite group, the monocyte was significantly decreased when compared with the control group ($p<0.05$).

3.1.4 Effect on the biochemical values

The results of biochemical examination of the female and male rats treated groups are summarized in table 4.16 and 4.17, respectively. In the female rats treated with 300 and 1,200 mg/kg body weight/day and in the satellite group, the concentration of direct bilirubin was significantly decreased when compared with that of the control group. In the female rats treated with 600 mg/kg body weight/day, aspartate aminotransferase (AST) was significantly increased. Furthermore, the satellite female group showed significant decreases in blood urea nitrogen (BUN), albumin, direct bilirubin, and aspartate aminotransferase (AST) concentrations as compared with the control values. In the male rats treated with 600 and 1,200 mg/kg body weight/day, the total protein was significantly increased in relation to the control group. In addition, the satellite male group showed significant decreases in albumin, direct bilirubin, and alkaline phosphatase concentrations as compared with the control values ($p<0.05$).

3.1.5 Effect on the histopathological values

The internal organs of all female and male rats such as lung, heart, liver, spleen, kidneys adrenals, sex organs, thymus, stomach, intestine, muscle, pancreas, brain and spinal cord were performed on gross and microscopic. The results also showed no significant histopathological change in the internal organs (Fig 4.1-4.30).

3.2 Chronic toxicity study of *T. bellerica*

3.2.1 Effect on the on the general behavior

After the rats were oral administered with the water extract from dried fruits of *T. bellerica*, all of the treated rats (300, 600 and 1,200 mg/kg body weight/day) were healthy as shown by the normal general behaviors, respiratory pattern, cardiovascular signs, motor activities, reflexes, and normal change in skin and fur (data not shown).

3.2.2 Effect on the body weight and organ weights

The body weight and body weight gain in treated groups of male and female rats are shown in table 4.18. In the female rats treated with all dose of the extracts, they had no significant differences in their body weight and body weight gain when compared with the control groups. In the male rats treated with 300 mg/kg body weight, the body weight and body weight gain showed significant decreases on day 270 as compared with the control group. In addition, the body weight and body weight gain of male rats treated with 600, 1200 mg/kg body weight/day and satellite group showed significant decreases on days 90, 180, and 270 when compared with the control group ($p < 0.05$).

The internal organ weights in treated groups of both male and female rats are shown in table 4.19 and 4.20, respectively. In the female rats treated with all doses of the extracts, there were no significant differences in the internal organ weight when compared with the control group. The male rats treated with 300 mg/kg body weight/day showed the heart weight significantly lower than that of the control. Furthermore, the male rats treated with 600 mg/kg body weight/day, had brain, lung, heart, liver and kidney weight significantly lower than those of the control. At the dose of 1,200 mg/kg body weight/day, a significant weight decrease was found in lung, heart and spleen when compared with the control. Besides, the satellite male group showed a significant increase in the kidney weight when compared with the control ($p<0.05$).

3.2.3 Effect on the hematological values

The results of hematological parameters of female and male rats were shown in table 4.21 and 4.22, respectively. In the female rats treated with various doses, the hematological parameters showed no significant difference when compared with the control. Furthermore, in the male rats treated with 600 mg/kg body weight showed significantly lower in the MCV than that of the control group ($p<0.05$).

The differential white blood cell count values of the female and male rats treated groups are shown in table 4.23 and 4.24, respectively. In the female rats, a significant increase in neutrophil and a significant decrease in lymphocyte were

observed in the satellite group. In the male rats, a significant increase in white blood cell was observed in the treated groups with 300 and 600 mg/kg body weight. The male rats treated with 600 mg/kg body weight/day, lymphocyte was significantly decreased when compared with the control group ($p<0.05$).

3.2.4 Effect on the biochemical values

The clinical blood chemistry examinations of the female and male rats are presented in table 4.25 and 4.26, respectively. In the female rats treated with 300 and 600 mg/kg body weight/day, the level of glucose was significantly increased when compared with the control group. In the satellite female group, it showed significant decreases in albumin, total bilirubin, direct bilirubin, and AST. Additionally, it was significantly increased in alkaline phosphatase concentration as compared with the control value. In the male rats treated with 1,200 mg/kg body weight/day showed a significant increase in total protein. In the satellite male group showed significant decreases in BUN, total bilirubin, and direct bilirubin concentrations as compared with the control values ($p<0.05$).

3.2.5 Effect on the histopathological values

The internal organs of all female and male rats such as lung, heart, liver, spleen, kidneys adrenals, sex organs, thymus, stomach, intestine, muscle, pancreas, brain and spinal cord were performed on gross and microscopic. The results also showed no significant histopathological change in the internal organs (Fig 4.31-4.60).

Table 4.1

Monograph of *T. chebula*

Physical appearance	The fruits are usually smooth or frequently 5-ridged, ellipsoid to ovoid drupes, and yellow to orange brown in color with a size of 2-3 x 3-4 cm. 
% Loss on drying	6.76
% Total ash	6.37
% Acid insoluble ash	2.61
% Extractive value	
Hexane extractive	1.31
Dichloromethane extractive	4.20
Ethanolic extractive	34.11
Water extractive	55.45
Chemical compounds screening	Flavonoids, hydrolysable tannin, terpenes, and blue-fluorescence compounds.
Microbial test	Total aerobic count $<9.0x10^2$ (regulation $<5.0x10^7$) Yeast and mold count $<2.0x10^2$ (regulation $<5.0x10^4$) Not found of Enterobacteriaceae, *Escherichia coli*, *Salmonella* sp., *Clostridium* sp., and *Staphylococcus aureus*
Aflatoxin test	Not found
Heavy metal test	Less than regulation (As 0.52; Cd 0.02; Hg 0.23; Pb 0.58 ppm)

Table 4.2

Monograph of the *T. chebula* extract

Physical appearance	The powder is brown color, characteristic odour, sour, slightly bitter and astringent. 
% Loss on drying	1.99
% Total ash	5.17
% Acid insoluble ash	0.31
UV spectrum	λmax 208, 264 nm
IR spectrum	3312, 2936, 1702, 1607, 1341, 1235, 1041, 929, 579
Chemical compounds screening	Flavonoids, hydrolysable tannin, terpenes, and blue-fluorescence compounds.
% Tannins	26.75
% Total carbohydrate	19.63
% Uronic acid	5.39
% Gallic acid	2.43
Microbial test	Total aerobic count $<5.0x10^2$ (regulation $<5.0x10^7$) Yeast and mold count <5.0x10 (regulation $<5.0x10^4$) Not found of Enterobacteriaceae, *Escherichia coli*, *Salmonella* sp., *Clostridium* sp., *Staphylococcus aureus*
Heavy metal test	Less than regulation (As 0.20; Cd 0.02; Hg 0.13; Pb 0.19 ppm)
% Yield of the extract in raw material	9.62
% Yield of the extract in spray dry	87.84

Table 4.3

Monograph of *T. bellerica*

Physical appearance	Fruit drupe, subglobose to ellipsoid, obscurely 5-angled with a size of 2.5 x 3.5 cm. 
% Loss on drying	8.27
% Total ash	5.42
% Acid insoluble ash	0.32
% Extractive value	
Hexane extractive	0.60
Dichloromethane extractive	1.83
Ethanolic extractive	12.81
Water extractive	70.99
Chemical compounds screening	Flavonoids, hydrolysable tannin, saponin, terpene, and blue-fluorescence compounds.
Microbial test	Total aerobic count $<2.9x10^3$ (regulation $<5.0x10^7$) Yeast and mold count <10 (regulation $<5.0x10^4$) Not found of Enterobacteriaceae, *Escherichia coli*, *Salmonella* sp., *Clostridium* sp., *Staphylococcus aureus*
Aflatoxin test	Not found
Heavy metal test	Less than regulation (As 0.26; Cd 0.03; Hg 0.02; Pb 0.18 ppm)

Table 4.4

Monograph of the *T. bellerica* extract

Physical appearance	The powder is dark brown color, characteristic odour, acid and astringent taste. 
% Loss on drying	3.76
% Total ash	9.14
% Acid insoluble ash	0.40
UV spectrum	λmax 218, 274 nm
IR spectrum	3288, 1712, 1610, 1343, 1204, 1058
Chemical compounds screening	Flavonoids, hydrolysable tannin, saponin, terpene, and blue-fluorescence compounds.
% Tannins	34.46
% Total carbohydrate	20.00
% Uronic acid	5.28
% Gallic acid	7.98
Microbial test	Total aerobic count $<10^3$ (regulation $<5.0x10^7$) Yeast and mold count $<10^3$ (regulation $<5.0x10^4$) Not found of Enterobacteriaceae, *Escherichia coli*, *Salmonella* sp., *Clostridium* sp., *Staphylococcus aureus*
Heavy metal test	Less than regulation (As 0.33; Cd 0.05; Hg 0.08; Pb 0.18 ppm)
% Yield of the extract in raw material	4.23

Table 4.5

Body weights of rats in the acute toxicity study of *T. chebula*

Group	Body weight (g)			
	Day 0	**Day 7**	**Day 14**	**Weight gain on day 14**
Female				
Control	132.00 ± 2.33	169.60 ± 2.38	189.20 ± 3.49	57.20 ± 2.50
T. chebula	131.80 ± 1.96	169.00 ± 2.27	189.20 ± 3.45	57.40 ± 2.78
Male				
Control	158.90 ± 2.98	217.70 ± 3.34	266.40 ± 2.62	107.60 ± 2.18
T. chebula	158.90 ± 3.80	216.60 ± 3.70	259.50 ± 8.14	100.60 ± 5.73

Values are expressed as mean ± S.E.M., n = 5

The test groups, received a single oral dose of 5,000 mg/kg body weight

There were no significant differences at $p<0.05$.

Table 4.6

Organ weights of rats in the acute toxicity study of *T. chebula*

Organ	Organ weights (g)	
	Control	***T. chebula* of 5,000 mg/kg body weight**
Female		
Lung	1.15 ± 0.03	1.11 ± 0.04
Heart	0.75 ± 0.02	0.79 ± 0.02
Liver	5.79 ± 0.11	5.51 ± 0.18
Spleen	0.56 ± 0.01	0.58 ± 0.02
Adrenal	0.03 ± 0.00	0.03 ± 0.00
Kidney	0.74 ± 0.01	0.74 ± 0.01
Ovary	0.06 ± 0.00	0.05 ± 0.00
Uterus	0.39 ± 0.02	0.33 ± 0.02
Brain	1.69 ± 0.01	1.73 ± 0.03
Male		
Lung	1.39 ± 0.03	1.32 ± 0.04
Heart	0.97 ± 0.02	1.01 ± 0.03
Liver	8.12 ± 0.12	7.81 ± 0.26
Spleen	0.75 ± 0.02	0.76 ± 0.02
Adrenal	0.03 ± 0.00	0.03 ± 0.00
Kidney	1.04 ± 0.02	1.03 ± 0.02
Testis	1.30 ± 0.06	1.43 ± 0.03
Epididymis	0.27 ± 0.01	0.27 ± 0.01
Brain	1.78 ± 0.02	1.75 ± 0.02

Values are expressed as mean ± S.E.M., n = 5

There were no significant differences at $p<0.05$.

Table 4.7

Body weights of rats in the acute toxicity study of *T. bellerica*

Group	Body weight (g)			
	Day 0	**Day 7**	**Day 14**	**Weight gain on day 14**
Female				
Control	131.50 ± 2.36	171.00 ± 3.03	190.20 ± 3.85	58.70 ± 3.61
T.bellerica	131.80 ± 1.31	167.00 ± 1.80	184.00 ± 2.68	53.20 ± 2.33
Male				
Control	161.80 ± 6.54	221.60 ± 6.71	263.30 ± 8.01	101.60 ± 6.15
T.bellerica	159.80 ± 3.31	216.00 ± 5.31	262.90 ± 6.77	103.10 ± 4.66

Values are expressed as mean ± S.E.M., n = 5

The test groups, received a single oral dose of 5,000 mg/kg body weight

There were no significant differences at $p<0.05$.

Table 4.8

Organ weights of rats in the acute toxicity study of *T. bellerica*

Organ	Organ weights (g)	
	Control	***T. bellerica* of 5,000 mg/kg body weight**
Female		
Lung	1.12 ± 0.03	1.08 ± 0.02
Heart	0.78 ± 0.02	0.73 ± 0.01
Liver	5.12 ± 0.51	5.44 ± 0.12
Spleen	0.60 ± 0.04	0.59 ± 0.03
Adrenal	0.03 ± 0.00	0.04 ± 0.00
Kidney	0.76 ± 0.01	0.67 ± 0.05
Ovary	0.05 ± 0.00	0.05 ± 0.00
Uterus	0.34 ± 0.02	0.31 ± 0.04
Brain	1.70 ± 0.02	1.65 ± 0.04
Male		
Lung	1.26 ± 0.06	1.29 ± 0.04
Heart	1.04 ± 0.04	1.04 ± 0.03
Liver	8.22 ± 0.37	8.70 ± 0.49
Spleen	0.80 ± 0.04	0.76 ± 0.02
Adrenal	0.03 ± 0.00	0.03 ± 0.00
Kidney	1.09 ± 0.03	1.11 ± 0.05
Testis	1.50 ± 0.02	1.34 ± 0.08
Epididymis	0.29 ± 0.01	0.27 ± 0.01
Brain	1.80 ± 0.02	1.67 ± 0.10

Values are expressed as mean ± S.E.M., n = 5

There were no significant differences at $p<0.05$.