

Acute Toxicity of Flower Extracts from *Dolichandrone serrulata* in Mice

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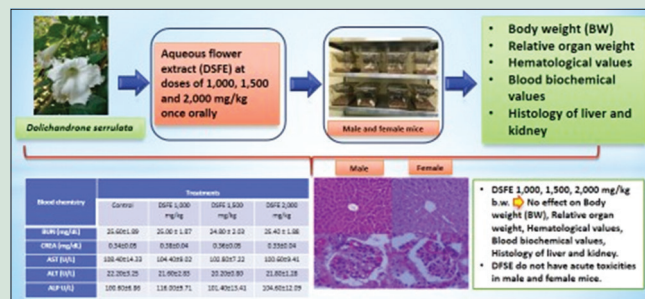
ABSTRACT

Context: *Dolichandrone serrulata* flowers are widely used as vegetables in northern and eastern Thailand. However, there is no report on the toxicities of this plant. **Objective:** The present study was aimed to determine the acute toxicity of aqueous flower extracts from *D. serrulata* in ICR mice. **Materials and Methods:** The extract at dose of 1000, 1500, and 2000 mg/kg was orally administered once to mice in order to investigate an acute toxicity. **Results:** The extract did not produce any sign or symptom of toxicity. Dead mouse was not found within the first 24 h and for further 14 days. The body weight increased in comparison to the controls. However, the relative organ weight between the treated and control mice was not different. The hematological values were not altered by the treatment of the extracts. The liver function parameters including aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase among treated mice were not different. The extract did not alter the kidney function parameters (blood urea nitrogen and creatinine). The lipid profiles in treated mice were not changed in comparison to the controls. In addition, histopathological features of the liver and kidney are not altered by the administration of the extracts. **Conclusion:** The results demonstrated that the maximum dose of *D. serrulata* flower extracts (2000 mg/kg) does not cause the acute toxicity in male and female mice.

Key words: Acute toxicity, aqueous extracts, *Dolichandrone serrulata*, flower, mice

SUMMARY

- There are no mortality and behavioral changes in mice treated with all doses (1000, 1500, and 2000 mg/kg body weight) of *Dolichandrone serrulata* flower extracts for 24 h and further 14 days. All treated groups have an increased body weight. The extracts do not have any effect on relative organ weight, hematological values, blood biochemical values, and histopathology of the liver and kidney. Therefore, the extracts do not cause the acute toxicity in male and female mice.



Abbreviations Used: DSFE: *Dolichandrone serrulata* flower extracts, b. w.: Body weight, WBC: White blood cell, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, LYMPH: Lymphocyte, MONO: Monocyte, BASO: Basophil, BUN: Blood urea nitrogen, CREA: Creatinine, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase.

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INTRODUCTION

Dolichandrone serrulata (DC.) Seem is a plant species which is belonging to the family Bignoniaceae. It is a deciduous tree to 25 m tall with narrow cylindrical crown. Flower is 12–21 cm, pure white, opening at night in short unbranched clusters of 3–7 flowers at end of twigs, 2–3 cm. Leaves are to 43 cm, odd-pinnate, 3–5 pairs of leaflets, 5–14 cm × 3–6 cm, elliptic with tapering tip and strongly asymmetric base, usually with scattered teeth. Fruit is up to 85 cm × 1.8 cm, pointed, spirally twisted. Bark is pale brown, smooth, or slightly flaking.^[1] As for the phytochemistry studies of this plant, Sinaphet *et al.* found a new phenolic triglycoside, dolichandroside, isolated from the branches of *D. serrulata* together with decaffeoyl-verbascoside, verbascoside, isoverbascoside, markhamioside A, 2-O-apiosylverbascoside, luteoside B, and ixoside.^[2] Phanthong *et al.* found that separation of *D. serrulata* flowers yielded six compounds, identified as hallerone, protocathechuic acid, rengyolone, cleroidicin B, ixoside, and isomaltose and the first report on hallerone, protocathechuic acid, rengyolone, cleroidicin B, and isomaltose in this plant.^[3] The biological screenings revealed that the highest antioxidant activity at 500 µg/ml using DPPH free radical scavenging method was demonstrated by *D. serrulata* leaf ethanolic extract followed by twigs, pods, seeds, and flowers, respectively. The highest antilipoxidase activity at 1 mg/ml was demonstrated by *D. serrulata* leaf extract followed

by twigs, seeds, pods, and flowers, respectively.^[4] *D. serrulata* flowers are widely used as vegetables in northern and eastern Thailand.^[5] However, there is no report on the toxicities of this plant. Therefore, the objectives of the present study were to study the acute toxicity of aqueous flower extracts from *D. serrulata* in ICR mice to evaluate the safety of using this plant as food or alternative medicine.

MATERIALS AND METHODS

Plant preparation and extraction

Flowers of *D. serrulata* were collected from the cultivation area in Mahasarakham Province, northeastern Thailand. The flowers were

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cleaned by tap water and dried in hot air oven at 50°C overnight. Then, they were ground as fine powders using an electrical grinder. The flower powders were refluxed. The *D. serrulata* flower extracts (DSFEs) were filtered using filter paper Whatman No. 1. The filtrates were then freeze-dried as fine powder and kept at -20°C until used.

Animal preparation

Male and female ICR mice at 50–70 g body weight (b. w.) were housed at the Experimental Animal Laboratory (Animal Biosafety Level 3; ABSL3), Northeastern Center for Laboratory Animals, Khon Kaen University, Thailand. They were housed in the animal room with temperature of 22°C ± 3°C and relative humidity of 88%. The dark-light cycle was 12 h dark and light. The drinking water and food pellets were provided *ad libitum*. The animal protocol was approved by the Animal Ethics Committee, Khon Kaen University, Thailand. The approval number is IACUC, KKU 99/61.

Experimental designs

The mice were divided into eight experimental groups as the following – Group 1: male normal controls; Group 2: male normal + 1000 mg/kg flower extracts; Group 3: male normal + 1500 mg/kg flower extracts; Group 4: male normal + 2000 mg/kg flower extracts; Group 5: female normal controls; Group 6: female normal + 1000 mg/kg leaf extracts; Group 7: female normal + 1500 mg/kg flower extracts; and Group 8: female normal + 2000 mg/kg flower extracts.

The flower extracts at doses of 1000, 1500, and 2000 mg/kg b. w. were administered once and orally to male and female mice. The behavioral changes were further observed for 14 days. During the experiments, the body weights of rats were measured weekly. At the end of the experiments, the mice were sacrificed by isoflurane inhalation, and the blood samples were collected for blood biochemical parameter investigation. The blood biochemical parameters include red blood cells, white blood cells, platelets, alkaline phosphatase, blood urea nitrogen, and creatinine using automatic blood chemical analyzer (BT 2000 Plus, Germany). The internal organs including liver, kidney, heart, spleen, and lung were removed and calculated for the relative organ weight by the following formula:

Relative organ weight = organ weight/body weight × 100

The tissues of the liver and kidney were washed with normal saline and immediately fixed with 10% formalin for histopathological study. The tissues were then dehydrated by the series of alcohols in the tissue processor machine. The dehydrated tissues were cut and placed in

cassettes. They were then embedded in paraffin and cut using rotary microtome with the thickness of 5–7 µm. The paraffin was removed from the tissues by placing in the warm water bath. The tissues were stained by hematoxylin and eosin and observed under the light microscope.

Statistical analysis

All data were expressed as mean ± standard error of the mean with *n* = 5. Comparisons of the difference of means were performed using one-way ANOVA. *P* < 0.05 was used as confidence interval.

RESULTS

Behavioral changes

The extracts at doses of 1000, 1500, and 2000 mg/kg b. w. did not affect the behavioral changes including reluctance to move, abnormal movement, loss of appetite, loss weight, as well as mortality in male and female mice, indicating that the extracts could not cause the acute toxicity in mice [Tables 1 and 2].

Body weight and relative organ weight

Regarding body weight and relative organ weight, the extracts did not affect the body weight and relative organ weight in mice. The body weights of mice in control and treated groups tended to be increased, suggesting that mice in all experimental groups have a normal food and water consumption [Tables 3 and 4]. In addition, the relative organ weight in the control and treated mice was not significantly different [Tables 5 and 6].

Hematological values

The hematological investigations in the acute toxicity of the extracts were carried out to confirm the effect of the extracts on cardiovascular systems in the animal model. It was found that the hematological parameters in control and treated mice were not statistically different. All hematological values were in the normal ranges [Tables 7 and 8].

Blood biochemical parameters

On the other hands, the blood biochemical parameters were not affected by the administration of all doses of the extracts to mice. These indicate that the liver and kidney of treated mice function normally. There is no sign of acute toxicity on hepatorenal functions in mice [Tables 9 and 10].

Table 1: Toxicity signs of male mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Treatment	Behavioral change				Mortality
	Reluctance to move	Abnormal movement	Loss of appetite	Loss weight	
Control	x	x	x	x	x
DSFE 1000 mg/kg	x	x	x	x	x
DSFE 1500 mg/kg	x	x	x	x	x
DSFE 2000 mg/kg	x	x	x	x	x

DSFE: *Dolichandrone serrulata* flower extracts; x: not found

Table 2: Toxicity signs of female mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Treatment	Behavioral change				Mortality
	Reluctance to move	Abnormal movement	Loss of appetite	Loss weight	
Control	x	x	x	x	x
DSFE 1000 mg/kg	x	x	x	x	x
DSFE 1500 mg/kg	x	x	x	x	x
DSFE 2000 mg/kg	x	x	x	x	x

DSFE: *Dolichandrone serrulata* flower extracts; x: not found

Table 3: Percentage increasing of body weight of male mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Treatments	Percentage increasing of body weight	
	Day 7	Day 14
Control	5.19±3.99	11.62±4.38
DSFE 1000 mg/kg	11.63±1.44	19.38±1.77
DSFE 1500 mg/kg	12.74±1.84	18.88±1.67
DSFE 2000 mg/kg	8.02±1.33	12.22±2.10

DSFE: *Dolichandrone serrulata* flower extracts**Table 4:** Percentage increasing of body weight of female mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Treatments	Percentage increasing of body weight	
	Day 7	Day 14
Control	4.99±1.39	9.59±1.26
DSFE 1000 mg/kg	6.49±1.54	8.47±5.57
DSFE 1500 mg/kg	4.99±1.39	9.59±1.26
DSFE 2000 mg/kg	5.34±3.41	12.04±3.03

DSFE: *Dolichandrone serrulata* flower extracts

Histopathological study of liver and kidney tissues

In the present study, the hepatocytes and central veins in the liver tissues of treated mice were normally arranged. No necrosis or lipid accumulation was found [Figures 1 and 2].

In addition, there is no change in the size of glomerulus and Bowman's capsule space in kidney tissues of treated mice. No necrosis was found in both renal corpuscle and renal tubule [Figures 3 and 4].

DISCUSSION AND CONCLUSION

Flower parts of *D. serrulata* have been used as food for people in the north and northeast of Thailand. However, the toxicity assessment has not been investigated for the safe consumption of this plant. It was found that once and orally administration of DSFE at doses of 1000, 1500, and 2000 mg/kg b. w. to the mice did not affect the behavioral changes. No dead animal was found during the experiments.

The extracts did not affect the blood biochemical values, hematological values, and relative organ weight. It might be implied that flower extracts of *D. serrulata* do not exhibit an acute toxicity in male and female mice.

These results were confirmed by histopathological study of liver and kidney tissues. The liver is an organ that involved in biotransformation

Table 5: Relative organ weight of male mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Relative organ weight (%)	Treatments			
	Control	DSFE 1000 mg/kg	DSFE 1500 mg/kg	DSFE 2000 mg/kg
Liver	4.359±0.102	4.570±0.115	4.592±0.114	4.302±0.116
Kidney	1.747±0.087	1.741±0.062	1.700±0.059	1.825±0.092
Heart	0.546±0.037	0.551±0.034	0.485±0.013	0.502±0.022
Lung	0.643±0.029	0.632±0.029	0.575±0.021	0.625±0.030
Spleen	0.398±0.022	0.325±0.031	0.309±0.020	0.375±0.040

DSFE: *Dolichandrone serrulata* flower extracts**Table 6:** Relative organ weight of female mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Relative organ weight (%)	Treatments			
	Control	DSFE 1000 mg/kg	DSFE 1500 mg/kg	DSFE 2000 mg/kg
Liver	3.728±0.076	3.619±0.271	3.896±0.221	4.025±0.3106
Kidney	1.074±0.061	0.977±0.038	1.192±0.070	1.146±0.080
Heart	0.486±0.025	0.373±0.028	0.426±0.029	0.409±0.042
Lung	0.584±0.051	0.573±0.050	0.524±0.020	0.566±0.030
Spleen	0.391±0.057	0.379±0.026	0.457±0.033	0.409±0.038

DSFE: *Dolichandrone serrulata* flower extracts**Table 7:** Hematological values of male mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

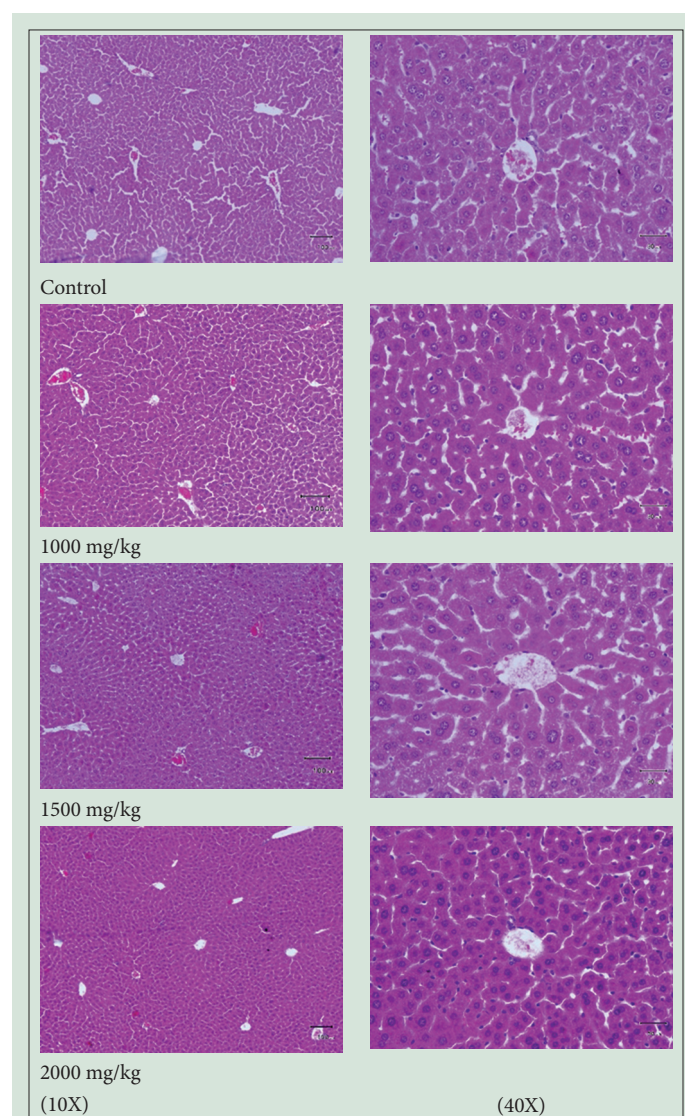
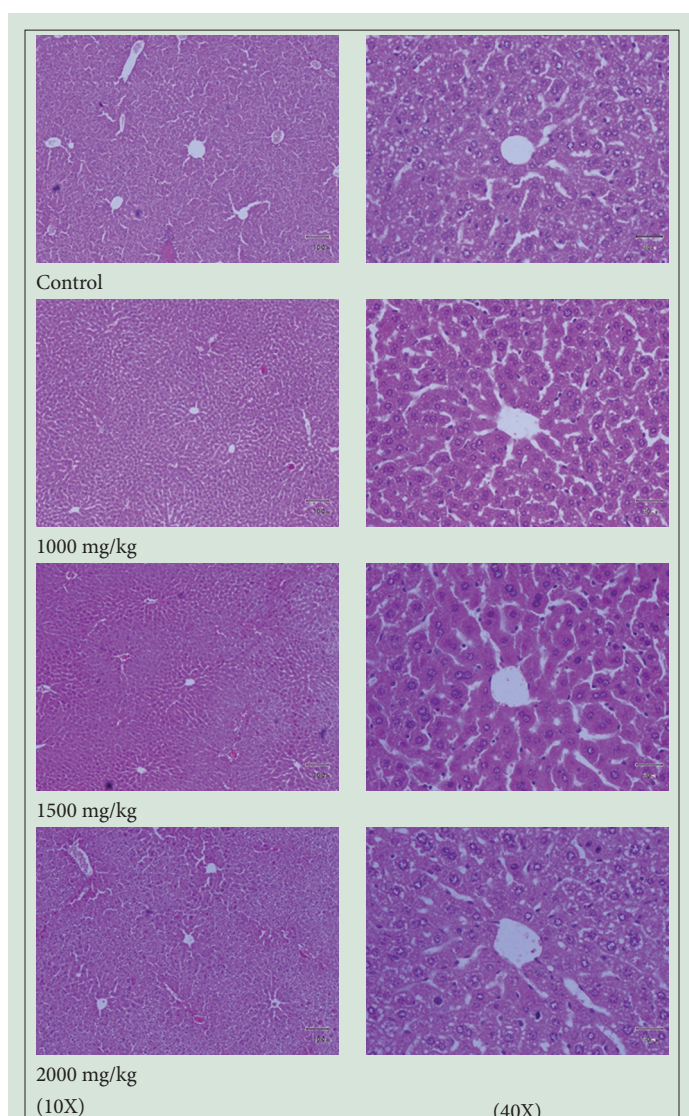
Hematological values	Treatments			
	Control	DSFE 1000 mg/kg	DSFE 1500 mg/kg	DSFE 2000 mg/kg
WBC (10 ³ /μl)	4.40±0.48	4.12±0.47	4.45±0.71	4.40±1.40
RBC (10 ³ /μl)	8.48±0.19	8.85±0.08	8.19±0.56	9.26±0.23
HGB (g/dL)	13.76±0.32	14.22±0.08	13.42±0.78	12.24±3.31
HCT (%)	44.10±0.70	45.22±0.06	42.13±2.54	48.17±0.71
MCV (fL)	51.50±1.16	52.50±0.14	51.26±0.46	51.88±0.73
MCH (pg)	16.02±0.46	16.40±0.13	15.90±0.29	13.49±3.19
MCHC (g/dL)	30.82±0.25	31.34±0.11	30.78±0.30	26.48±5.44
PLT (10 ³ /μl)	1179.80±89.59	1009.60±81.85	1066.2±81.42	1082.40±86.81
LYMPH (10 ³ /μl)	88.58±2.42	78.28±3.82	92.00±0.74	92.30±0.67
MONO (10 ³ /μl)	3.14±0.56	1.22±0.06	1.80±0.31	1.42±1.77
BASO (10 ³ /μl)	2.16±0.48	1.92±0.26	2.98±0.28	2.96±0.22

WBCs: White blood cells; RBCs: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: MCH concentration; PLT: Platelet; LYMPH: Lymphocyte; MONO: Monocyte; BASO: Basophil; DSFE: *Dolichandrone serrulata* flower extracts

Table 8: Hematological values of female mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Hematological values	Treatments			
	Control	DSFE 1000 mg/kg	DSFE 1500 mg/kg	DSFE 2000 mg/kg
WBC ($10^3/\mu\text{l}$)	3.09 $\pm$ 0.41	3.26 $\pm$ 0.40	3.26 $\pm$ 0.45	3.42 $\pm$ 0.24
RBC ($10^3/\mu\text{l}$)	8.79 $\pm$ 0.12	8.70 $\pm$ 0.03	8.69 $\pm$ 0.41	8.66 $\pm$ 0.11
HGB (g/dL)	14.36 $\pm$ 0.08	15.00 $\pm$ 0.13	14.08 $\pm$ 0.50	14.28 $\pm$ 0.06
HCT (%)	46.78 $\pm$ 0.43	46.40 $\pm$ 0.14	45.78 $\pm$ 0.88	45.76 $\pm$ 0.55
MCV (fL)	53.00 $\pm$ 0.78	52.78 $\pm$ 0.29	50.68 $\pm$ 0.50	52.86 $\pm$ 0.10
MCH (pg)	16.16 $\pm$ 0.18	17.06 $\pm$ 0.22	16.00 $\pm$ 1.51	16.48 $\pm$ 0.17
MCHC (g/dL)	30.60 $\pm$ 0.29	32.20 $\pm$ 0.18	31.52 $\pm$ 0.84	31.20 $\pm$ 0.31
PLT ($10^3/\mu\text{l}$)	958.00 $\pm$ 66.22	1056.80 $\pm$ 62.33	1060.00 $\pm$ 64.17	1060.60 $\pm$ 61.77
LYMPH ($10^3/\mu\text{l}$)	91.12 $\pm$ 0.67	87.86 $\pm$ 1.82	90.34 $\pm$ 0.35	88.38 $\pm$ 2.16
MONO ($10^3/\mu\text{l}$)	1.62 $\pm$ 0.24	1.34 $\pm$ 0.46	3.34 $\pm$ 0.09	2.60 $\pm$ 0.71
BASO ($10^3/\mu\text{l}$)	1.80 $\pm$ 0.41	2.62 $\pm$ 0.17	2.98 $\pm$ 0.17	2.56 $\pm$ 0.54

WBCs: White blood cells; RBCs: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: MCH concentration; PLT: Platelet; LYMPH: Lymphocyte; MONO: Monocyte; BASO: Basophil; DSFE: *Dolichandrone serrulata* flower extracts

**Figure 1:** Histopathology of the liver in control and treated male rats ($\times 10$ and $\times 40$, scale bar = 10 and 30 μm , respectively)**Figure 2:** Histopathology of the liver in control and treated female rats ($\times 10$ and $\times 40$, scale bar = 10 and 30 μm , respectively)

of toxic substances in mammals. Moreover, the kidney is an organ that related to the toxic elimination in toxicokinetic process. It is found that there is no abnormality or lesion in the liver and kidney

tissues of treated mice. The results indicate that the flower extracts from *D. serrulata* do not possess any toxicity on the target organs in toxicokinetic process.

Table 9: Blood chemistry parameters of male mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

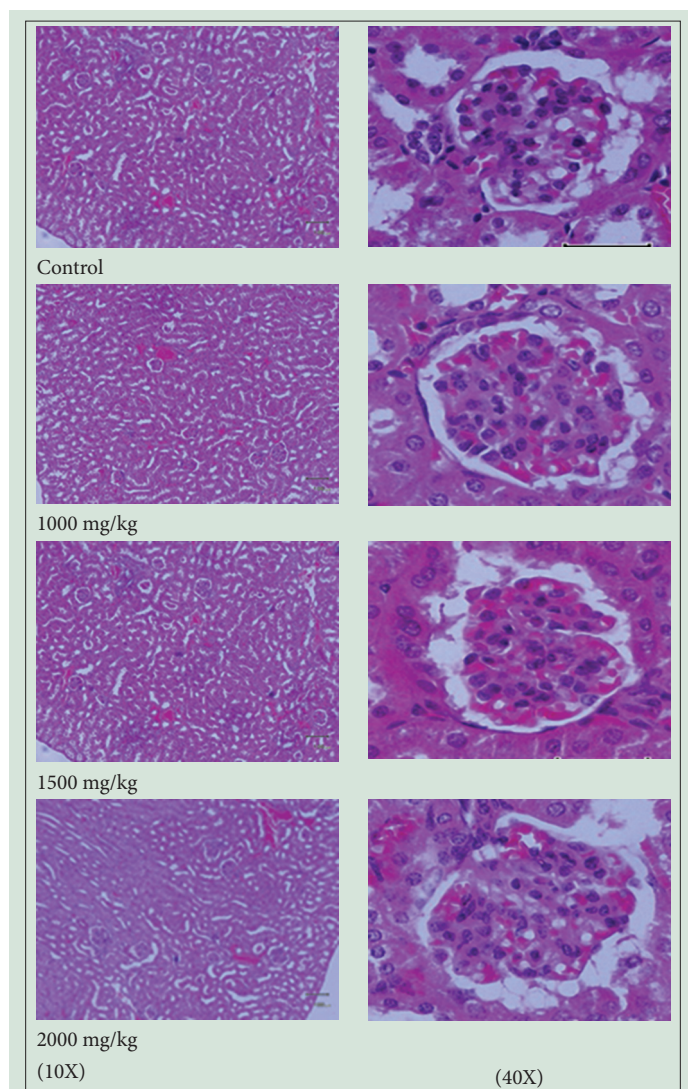
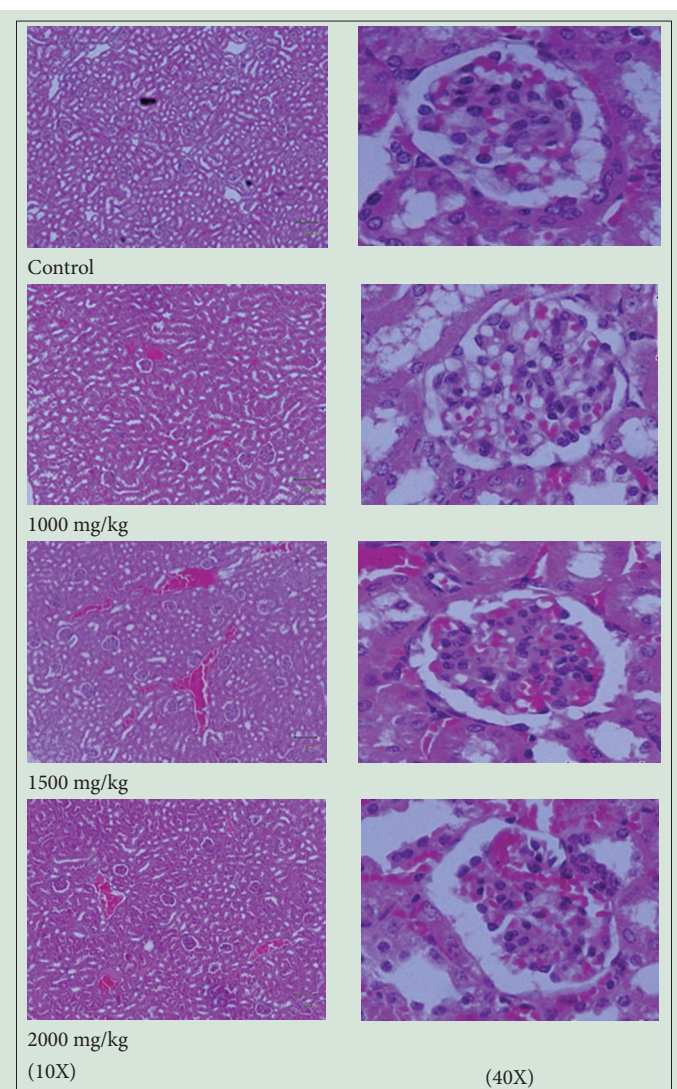
Blood chemistry	Treatments			
	Control	DSFE 1000 mg/kg	DSFE 1500 mg/kg	DSFE 2000 mg/kg
BUN (mg/dL)	25.60±1.89	25.00±1.87	24.80±2.03	25.40±1.88
CREA (mg/dL)	0.34±0.05	0.38±0.04	0.36±0.05	0.33±0.04
AST (U/L)	108.40±14.33	104.40±9.02	102.80±7.22	100.60±9.41
ALT (U/L)	22.20±3.25	21.60±2.83	20.20±0.80	21.80±1.28
ALP U/L)	100.60±6.86	116.00±9.71	101.40±13.41	104.60±12.09

BUN: Blood urea nitrogen, CREA: Creatinine; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; DSFE: *Dolichandrone serrulata* flower extracts

Table 10: Blood chemistry parameters of female mice treated once and orally with the flower extracts at doses of 1000, 1500, and 2000 mg/kg body weight

Blood chemistry	Treatments			
	Control	DSFE 1000 mg/kg	DSFE 1500 mg/kg	DSFE 2000 mg/kg
BUN (mg/dL)	23.00±2.36	22.00±3.85	29.00±3.15	25.20±2.35
CREA (mg/dL)	0.42±0.07	0.46±0.04	0.40±0.03	0.47±0.04
AST (U/L)	108.40±15.30	117.00±11.46	105.00±16.03	101.00±9.58
ALT (U/L)	25.20±2.76	23.20±3.33	22.40±2.88	23.80±4.86
ALP (U/L)	105.00±9.87	112.60±13.90	101.80±11.56	100.60±6.20

BUN: Blood urea nitrogen, CREA: Creatinine; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; DSFE: *Dolichandrone serrulata* flower extracts

**Figure 3:** Histopathology of the kidney in control and treated male rats (×10 and ×40, scale bar = 10 and 30 μm, respectively)**Figure 4:** Histopathology of the kidney in control and treated female rats (×10 and ×40, scale bar = 10 and 30 μm, respectively)

These results were supported by the previous phytochemical constituents and biological activities of this plant. Sinaphet *et al.* reported a new phenolic triglycoside, dolichandroside, from the branches of *D. serrulata*. Phanthong *et al.* firstly reported the phytochemicals including hallerone, protocatechuic acid, rengyolone, cleroidicin B, and isomaltose in the flower extracts of *Dolichandrone serrulata* with a good anti-oxidant activities. The flower parts of this plant are edible with high nutritive values, especially carbohydrate and energy.^[6] Moreover, other biological activities of this plant were reported such as antimicrobial^[7-9] and antipyretic activities.^[10] It could be implied that the secondary metabolites found in the flower extracts of *D. serrulata* are not toxic substances.

It can be concluded that the maximum dose of DSFE (2000 mg/kg) does not cause the acute toxicity in male and female mice.

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Conflicts of interest

There are no conflicts of interest.

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