

Original Article

## Toxicity evaluation of *Dolichandrone serrulata* flower extract on vital and reproductive organs in adult male rats

Avaliação da toxicidade do extrato da flor de *Dolichandrone serrulata* em órgãos vitais e reprodutivos em ratos machos adultos

C. Chaimontri<sup>a</sup>  and S. Iamsaard<sup>a,b,\*</sup> 

<sup>a</sup>Khon Kaen University, Faculty of Medicine, Department of Anatomy, Khon Kaen, Thailand

<sup>b</sup>Khon Kaen University, Research Institute for Human High Performance and Health Promotion, Khon Kaen, Thailand

### Abstract

Although *Dolichandrone serrulata* flower (DSF) aqueous extract has been shown to possess pharmacological properties, its systemic toxicity has still to be evaluated. The present study aimed to investigate the sub-chronic toxicity effect of DSF extract on biochemical parameters and histological structures of liver, kidney, testis, and epididymis plus vas deferens. Adult male rats were administered DSF at 100, 300, and 600 mg/kgBW via oral gavage for 48 consecutive days while control rats received distilled water. At the end of the experiment, blood, liver, kidney, testis, and epididymis plus vas deferens samples were collected to determine any changes to serum biochemical components including ALT, ALP, and creatinine levels and histological structures. The results revealed no significant difference in body weight and food or water consumption between control and the DSF-treated groups. It was found that DSF significantly increases the weight of epididymis plus vas deferens, while the kidney and liver showed a decrease in the high dose group ( $P$  value < 0.05). Histological changes in these vital and reproductive tissues including fibrosis were not observed after administration but ALT, ALP, and creatinine levels were significantly altered in the treated groups ( $P$  value < 0.05). These altered levels, however, were still within normal ranges. In conclusion, these findings demonstrated that *D. serrulata* flower extract had no sub-chronic toxicity on vital and reproductive structures but slightly altered some liver and kidney functions.

**Keywords:** *Dolichandrone serrulata*, liver, kidney, testis, epididymis.

### Resumo

Como o extrato aquoso da flor de *Dolichandrone serrulata* (DSF) demonstrou ter algumas propriedades farmacológicas, sua toxicidade sistêmica ainda não foi avaliada. Este estudo teve como objetivo investigar o efeito da toxicidade subcrônica do extrato de DSF em parâmetros bioquímicos e estruturas histológicas do fígado, rim, testículo e epidídimo mais os vasos deferentes. Ratos machos adultos foram administrados com DSF em 100, 300 e 600 mg / kg de peso corporal por meio de gavagem oral por 48 dias consecutivos, enquanto os ratos controle receberam água destilada. No final do experimento, amostras de sangue, fígado, rim, testículo e epidídimo mais canais deferentes foram coletados para determinar as alterações dos componentes bioquímicos séricos, incluindo ALT, ALP e níveis de creatinina e estruturas histológicas. Os resultados revelaram que o peso corporal e o consumo de comida ou água do controle e de todos os grupos tratados com DSF não foram significativamente diferentes. Verificou-se que o DSF aumentou significativamente o peso do epidídimo mais os canais deferentes, mas o rim e o fígado diminuiram no grupo de alta dose ( $P$  valor < 0,05). As alterações histológicas, incluindo fibrose de tais tecidos vitais e reprodutivos, não foram encontradas após a administração, mas os níveis de ALT, ALP e creatinina foram significativamente alterados nos grupos tratados (valor  $P$  < 0,05). No entanto, seus níveis alterados ainda estavam em intervalos normais. Em conclusão, esses achados demonstraram que o extrato da flor de *D. serrulata* não apresentou toxicidade subcrônica nas estruturas vitais e reprodutivas, mas alterou ligeiramente algumas funções hepáticas e renais.

**Palavras-chave:** *Dolichandrone serrulata*, fígado, rim, testículo, epidídimo.

## 1. Introduction

The use of herbs for the treatment of diseases is a long-standing tradition. The potential medicinal properties of plants depend on the types and amounts of their chemical

compounds. It is known that each part of individual medicinal plants has different compounds. Additionally, the type and quantity of substances can vary depending

\*e-mail: sattia@kku.ac.th

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on other factors such as the herb species, planting environment, and harvesting period (Sringeunyan and Hongwiset, 1990). Currently, Thailand's government is promoting the use of medicinal plants and herbs, which are considered medicinal alternatives to modern health care (Sringeunyan and Hongwiset., 1990) given that plant products are mostly nontoxic. Moreover, herbal treatments come from local knowledge in Thai traditional medicine. Khae Na or *Dolichandrone serrulata* (or *D. serrulata* flower (DSF)) is an ancient medicinal plant that has been traditionally believed to have many pharmacological properties (Sriprasert, 2016). Most parts (flower, pod, bark, and root) of this plant are useful and are consumed for their numerous pharmacological properties (Sriprasert, 2016; Kiratipaiboon et al., 2012). A current study has revealed the protective effect of DSF against reproductive damage in diabetic rats (Yannasithon et al., 2021). Biological studies of the methanolic extract of DSF have demonstrated its promising antioxidant activities (Phanthong et al., 2015). It was found that DSF extracts contained six compounds including hallerone, protocatechuic acid, renygolone, cleroidicin B, ixoside, and isomaltose (Phanthong et al., 2015). Moreover, a previous study has evaluated the phytochemical profiles and biological activities of DSF ethanolic extracts (Sriprasert, 2016). Phytochemical studies of the ethanolic extracts from this plant revealed secondary metabolites such as flavonoids, coumarins, saponins, tannins, terpenoids, steroids, and cardiac glycoside (Sriprasert, 2016). Recently, the aqueous extract of DSF has been reported to have antioxidant capacity and monoterpenoids such as renygolone and cleroidicin B (Chaimontri et al., 2021). However, some plants related to this *D. serrulata* have been shown to be toxic for vital and reproductive organs. For examples, *P. alliaceum* aqueous extract, extracted with ethanol in an acute toxicity study reduced white blood cell ability (Granados-Echegoyen et al., 2015). Previously, Aba and Amadi (2019) reported that the *A. carambola* fruit extract could damage hepatic and renal tissues. Additionally, it was found that sun ginseng could alter blood serum parameters including ALT, ALP, and creatinine levels, which indicate liver and kidney dysfunctions (Kim et al., 2013). Moreover, Azu et al. (2010) reported that high doses of *K. Africana* fruit extract could inhibit spermatogenesis. It was noted that the continuous consumption of recent plant products over an extended period can damage vital and reproductive organs (Kiratipaiboon et al., 2012; Freitas et al., 2013; Joshi et al., 2011; Karnati et al., 2013; Lee et al., 2012). Although DSF has been consumed for a long time, the toxic effects of its aqueous extract on liver, kidney, testis, and epididymis have yet to be assessed. To increase our understanding of this question, the present study sought to investigate the sub-chronic toxicity of DSF extracts on vital and reproductive organs to determine the possibility of safely consuming this substance over extended periods.

## 2. Materials and Methods

### 2.1. Animals

Forty adult male Sprague-Dawley rats (120-150g) were purchased from Nomura Siam International,

Bangkok, Thailand. Animals were kept in stainless-steel cages under experimental room conditions (12 hr light/dark cycle, temperature  $23 \pm 2$  °C, humidity 40-60%, sound < 85 decibels, and light intensity approximately 350-400 lux) in the Northeast Laboratory Animal Center, Khon Kaen University, Thailand. This experimental study was approved by the Animal Ethics Committee Research of Northeast Laboratory Animal Center, Khon Kaen University. The code of this animal ethic is IACUC-KKU-111/62.

### 2.2. Collection and preparation of *D. serrulata* flower

The plant specimens of fresh Khae Na were collected from the Ban Thum Subdistrict, Mueang District, Khon Kaen Province, Thailand, and authenticated by Assist. Prof. Dr. Pimwadee Pornponggrueng, Department of Biology, Faculty of Science, Khon Kaen University (voucher samples of *D. serrulata*, kept in the KKU Herbarium with a number of S. Iamsaard 02). In extraction, the dried DSF was sliced into small pieces prior to being soaked and boiled in distilled water at 90-95 °C for 40 min. The aqueous DSF extract was lyophilized using the lyophilizer (M-00359-1/57, 13-1011-18-2019, Labconco, Becthai Scientific Industry, Thailand). The percentage yield of the crude DSF extract was 40.78%. This extract was obtained from Dr. Sitthichai Iamsaard as previously described in the extraction details (Chaimontri et al., 2021; Yannasithon et al., 2021).

### 2.3. Experimental design

After acclimatization for 7 days, the male rats were randomly divided into 4 groups (10 rats per group) including control, DSF100, DSF300, and DSF600 groups, respectively. The DSF extract doses were calculated based on the maximal consumption dose of DSF in a person with an average weight of 60 kg. Briefly, the 600 mg/Kg BW dose was calculated from the consumption ability of 40 flowers/day/ person. The proportional ratios of the middle (300 mg) and lowest (100 mg) doses were calculated from 600 mg. For the DSF treated groups, the rats were treated with DSF-extract dissolved in distilled water (DW) at doses of 100, 300, and 600 mg/kg BW, respectively, via oral gavage. Animals in the control group were only given DW. Their body weights were recorded daily during administration over 48 consecutive days.

### 2.4. Biochemical analysis

At the end of the experiment, all rats were anesthetized with thiopental sodium (60 mg/kgBW via i.p.) before euthanasia by cervical dislocation. After opening the thoracic wall, blood was collected by cardiac puncture and centrifuged at 12,000 r/minute for 10 minutes at 4°C, using microcentrifuge (Microfuge 22R Centrifuge, Beckman Coulter TM, USA) to separate the blood cells from the serum. The blood serum was immediately sent to the immunology unit, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand for determination of blood urea nitrogen (BUN) and creatinine (for kidney functions) and albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (for liver functions). The biochemical levels were determined by using an electrochemiluminescence immunoassay

(Roche Diagnostics) with the cobas and immunoanalyzer (Roche Diagnostics, Mannheim, Germany).

### 2.5. Histological study of vital organs

After blood collection, the liver, kidney and reproductive organs including testis and epididymis were quickly collected and weighed. All tissues were fixed with 10% formalin fixative solution for 72 hrs. Then, they were processed for routine paraffin sections and stained with Masson's trichrome dyes to investigate the collagen fiber development. Briefly, the tissue sections were deparaffinized with xylene and rehydrated with serial descending alcohols. Then, the sections were immersed in Bouin's solution and stained with Weigert's iron hematoxylin. Subsequently, the sections were stained with Masson's trichrome staining kits (Catalogue no. HT15, Sigma-Aldrich, Inc., USA). Finally, the sections were washed with 1% acetic acid. The histological changes of vital organs and reproductive organs were observed and photographed by Nikon light ECLIPSE E200 microscope equipped with a DXM1200 digital camera.

### 2.6. Statistical analysis

All data were expressed as mean  $\pm$  standard deviation (SD). To compare the differences between the four groups, Post Hoc multiple comparisons were used for normally distributed - data and the differences of data were performed by using One Way ANOVA using program SPSS statistics 19.0.2 software (Statistical Package for the Social Sciences, version 19.0.2, SPSS Inc, Armonk, New York, USA.,

installed from KKU Software Center, Khon Kaen University) for data analysis. The SPSS program was used to examine the significant difference between groups. The *p*-value < 0.05 was considered as significant difference.

## 3. Results

### 3.1. Effect of *D. serrulata* flower extract on body weight, and vital and reproductive organs

The mean body, changed body, and testis weights of animals in the control and treatment groups showed no significant difference. By contrast, the weight of epididymis plus vas deferens in DSF600 treated group was significantly increased when compared with the control. Water intake and food consumption for all animals showed no significant variation (Table 1). Interestingly, the relative weights of kidney and liver in DSF300 and 600 treated animals were significantly decreased when compared to the control ( $P < 0.05$ ).

### 3.2. Effect of *D. serrulata* flower extract on biochemical parameters

After serum analyses, the biochemical parameters indicating liver function showed that DSF significantly decreased alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels compared to the control group ( $P < 0.05$ , Table 2). By contrast, creatinine levels which indicate kidney function, of the DSF100 and DSF300 groups

**Table 1.** Comparisons of body weight, testis, epididymis plus vas deferens, kidney, and liver organ weights between control and DSF-treated rats after 48 days of sub-chronic toxicity study.

Parameters	Groups			
	Control	DSF100	DSF300	DSF600
<b>Body weight (g)</b>	535.25 $\pm$ 18.34	534.25 $\pm$ 23.03	558.57 $\pm$ 21.23	550.50 $\pm$ 27.06
<b>Changed body weight (%)</b>	200.96 $\pm$ 9.81	193.44 $\pm$ 19.63	206.89 $\pm$ 14.63	202.26 $\pm$ 19.91
<b>Water intake/animal (ml)</b>	330.4	323.85	346.95	345.75
<b>Food consumption/animal (g)</b>	250.76	239.45	253.14	263.98
<b>Testis</b>				
Absolute weight (g)	1.753 $\pm$ 0.080	1.733 $\pm$ 0.130	1.815 $\pm$ 0.169	1.870 $\pm$ 0.140
Relative weight (g/100 g)	0.327 $\pm$ 0.015	0.324 $\pm$ 0.024	0.325 $\pm$ 0.030	0.339 $\pm$ 0.025
<b>Epididymis plus Vas deferens</b>				
Absolute weight (g)	0.614 $\pm$ 0.017	0.614 $\pm$ 0.028	0.659 $\pm$ 0.024	0.702 $\pm$ 0.056*
Relative weight (g/100 g)	0.114 $\pm$ 0.003	0.115 $\pm$ 0.005	0.118 $\pm$ 0.004	0.127 $\pm$ 0.010*
<b>Kidney</b>				
Absolute weight (g)	1.892 $\pm$ 0.040	1.814 $\pm$ 0.110	1.832 $\pm$ 0.080	1.837 $\pm$ 0.055
Relative weight (g/100 g)	0.353 $\pm$ 0.007	0.339 $\pm$ 0.020	0.328 $\pm$ 0.014*	0.333 $\pm$ 0.010*
<b>Liver</b>				
Absolute weight (g)	19.713 $\pm$ 1.277	18.187 $\pm$ 1.427	18.167 $\pm$ 1.165	18.155 $\pm$ 1.372
Relative weight (g/100 g)	3.683 $\pm$ 0.238	3.404 $\pm$ 0.267	3.252 $\pm$ 0.208*	3.298 $\pm$ 0.249*

Data were represented as mean  $\pm$  standard deviation (S.D.). \*Significant difference ( $P < 0.05$ ), compared with the control group. DSF = *Dolichandrone serrulata* flower extract.

**Table 2.** Comparisons of biochemical parameter levels in serum between control and treated rats after 48 days of sub-chronic toxicity study.

Biochemical parameters	Groups			
	Control	DSF100	DSF300	DSF600
<b>Liver functions</b>				
AST (U/L)	70.00 ± 0.00	92.00 ± 11.27	84.33 ± 12.74	78.00 ± 1.00
ALT (U/L)	30.33 ± 0.58	26.00 ± 0.00*	26.67 ± 1.53*	21.33 ± 0.58*
ALP (U/L)	136.00 ± 1.73	128.00 ± 1.00*	126.67 ± 2.52*	112.00 ± 1.73*
Albumin (g/dL)	3.97 ± 0.06	3.83 ± 0.32	4.00 ± 0.26	4.07 ± 0.06
<b>Kidney functions</b>				
BUN (mg/dL)	20.37 ± 0.12	21.77 ± 1.24	22.37 ± 0.93	21.60 ± 0.00
Creatinine (mg/dL)	0.30 ± 0.01	0.37 ± 0.01*	0.38 ± 0.02*	0.33 ± 0.001

Data were represented as mean ± standard deviation (S.D.). \*Significant difference ( $P < 0.05$ ), compared with the control group. AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; BUN = blood urea nitrogen.

were significantly increased when compared with the control group ( $P < 0.05$ , Table 2). Interestingly, no difference in renal function indicated by aspartate aminotransferase (AST), albumin, and blood urea nitrogen (BUN) levels in all DSF treated groups was observed.

### 3.3. Effect of *D. serrulata* flower extract on collagen fiber development in the testis, caudal epididymis, kidney, and liver of rats

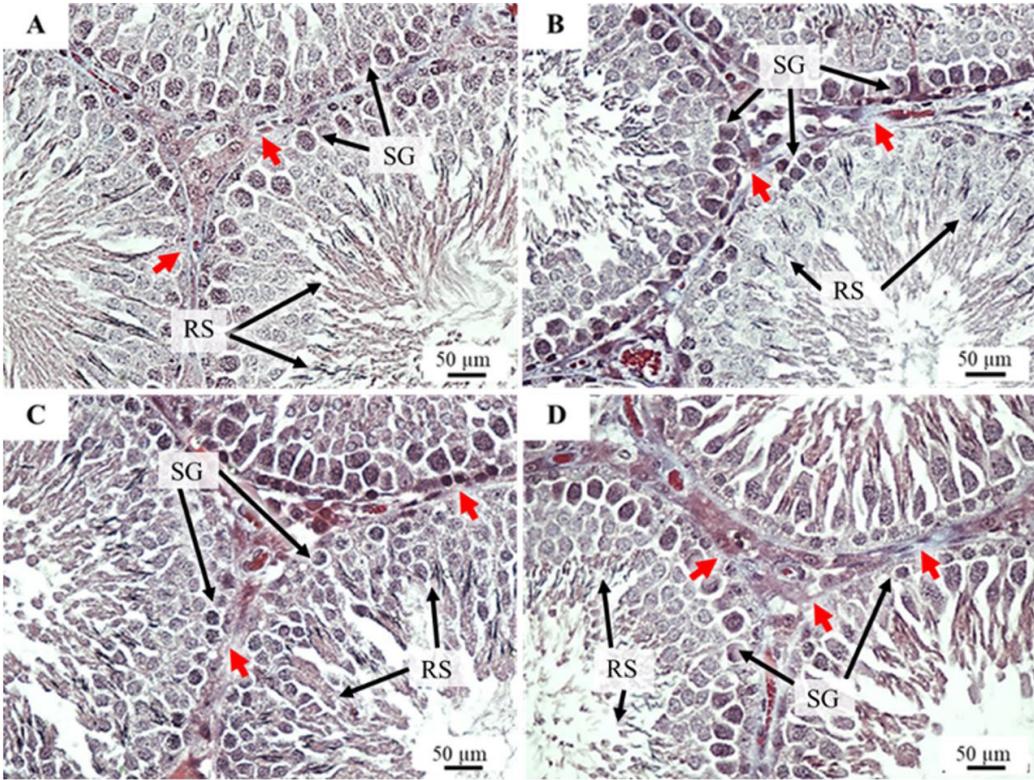
The tissue sections of testis, caudal epididymis, kidney, and liver stained by Masson's trichrome showed no difference in collagen fiber thickness among control and treated groups as shown in Figures 1–4. By contrast, abundant collagen fibers and fibroblasts were found at basal lamina underneath the seminiferous epithelium (blue arrows of Figure 1). In addition, the caudal epididymis shows fine collagen fibers along the tubular wall and inter tubular spaces (black stars in Figure 2). In the kidney, collagen fibers are present in the basement membrane of renal tubules and Bowman's space (blue arrows in Figure 3). Moreover, the liver tissue of all groups showed a small amount of collagen fiber surrounding the portal triad containing the hepatic artery, portal vein, and bile duct (blue arrows in Figure 4).

## 4. Discussion

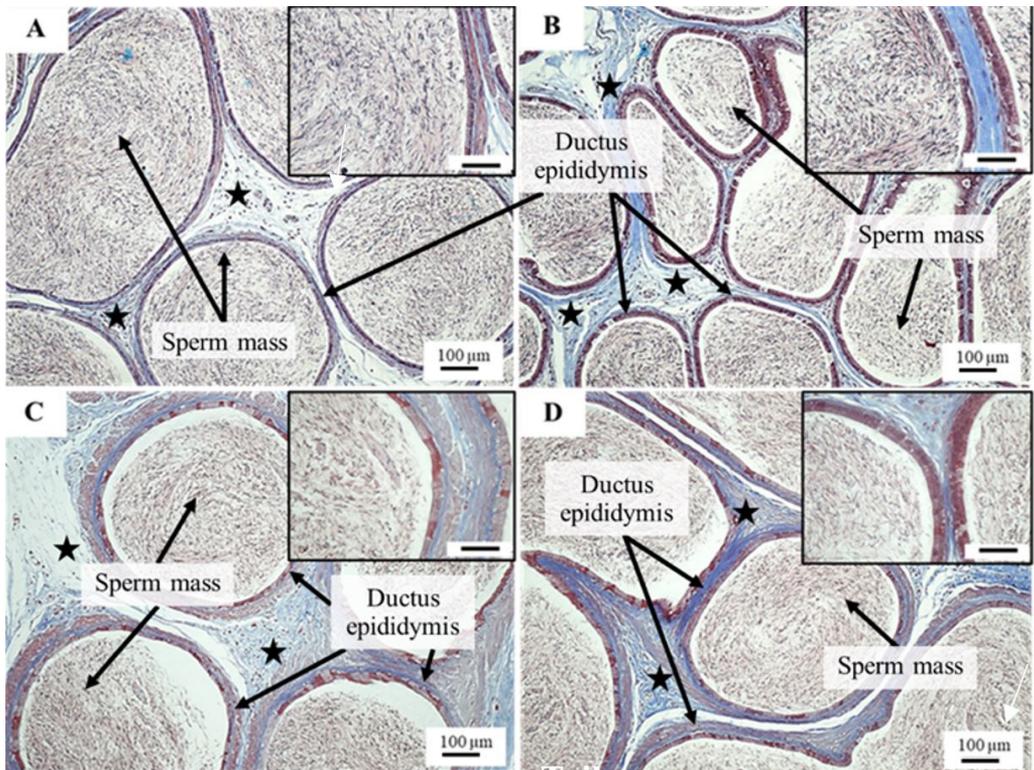
This study showed that DSF extract did not affect liver, kidney, and reproductive organ structures after administration for 48 days. However, DSF slightly altered liver and kidney function, but their serum levels were still within normal range in line with standard values. It has been reported that high doses (500 mg/KgBw) of the same species of this plant suppressed sperm production (Azu et al., 2010); however, a high dose of DSF (600 mg/KgBw) did not alter reproductive organ weight or histological structures. This result agreed with a previous study showing increased epididymal sperm concentration and quality after DSF treatment (Chaimontri et al., 2021). Moreover, DSF improved sperm count and physiology as

well as testicular histopathology in type 1 diabetic rats (Yannasithinon et al., 2021). Together with our results, those investigations suggest that aqueous DSF extract has no sub-chronic toxicity on the male reproductive system. This could be explained due to aqueous DSF presenting antioxidant activities and containing total phenolic contents including terpenoid components (Chaimontri et al., 2021). Indeed, in the literature, many parts of *D. serrulata* extracted by ethanol or methanol have also been shown to have antioxidant capacities and substances such as alkaloids, flavonoids, glycosides, phenolic compounds, saponins, and tannins (Chatchanayuenyong and Sujayanont, 2020; Khaing et al., 2018; Kiratipaiboon et al., 2012; Sinaphet et al., 2006; Sittiwet, 2009). These antioxidant capacities of DSF could decrease high fast blood glucose (FBG) levels and testicular malondialdehyde (MDA) with increased testosterone levels of Type 2 DM-treated rats (Yannasithinon et al., 2021).

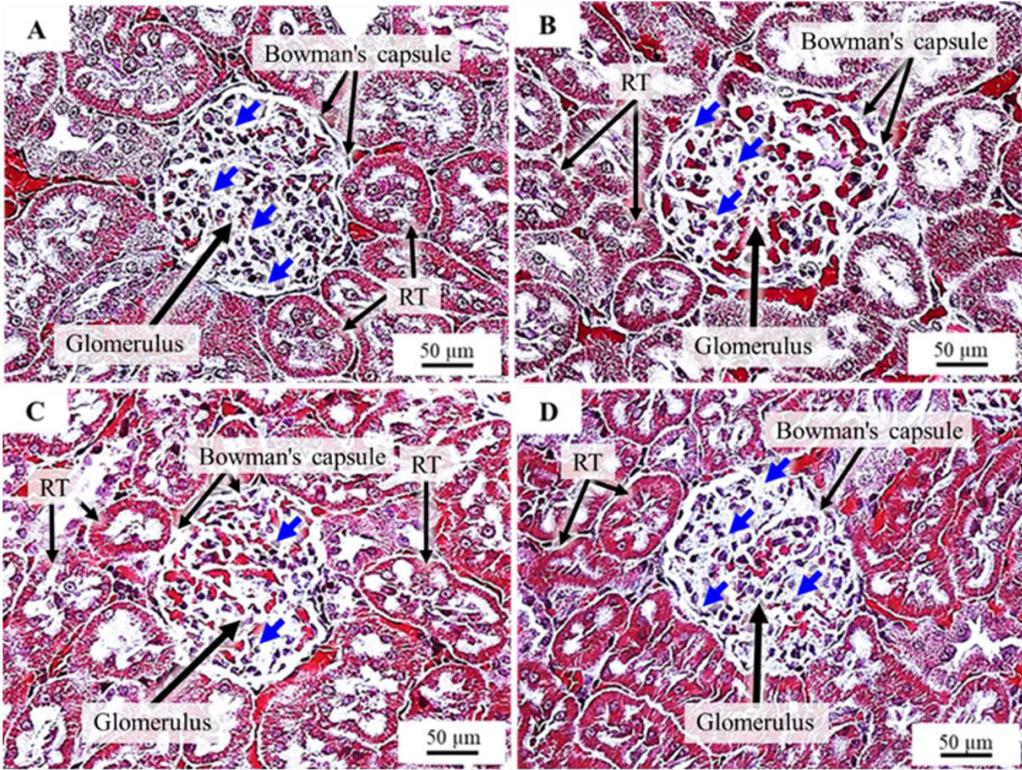
Previous studies have reported the toxicity of many substances including plant extracts on the collagen fiber deposits in diabetic testis (Ismail et al., 2017), hypertensive epididymis (Mazen and Zidan, 2017), hepatic stellate cell distribution (Nishimura et al., 2012; Towne et al., 2015), and chronic kidney disease patients (Allon et al., 2019). As demonstrated in this recent study, the intensity of collagen fibers present in testis, caudal epididymis, liver, and renal tissues stained by Masson's trichrome, shows no difference between the control and DSF treatment groups. These results indicated that DSF extract did not induce histopathological fibrosis, accumulated collagen fibers, in the vital and reproductive tissues. These results suggested that consuming DSF extract for a sub-chronic period did not cause fibrosis in reproductive or vital organs. It was noted that the thickness of the Bowman's capsule among DSF treated groups showed no obvious alterations in comparison with the control group. In addition, the weights of those organs were not significantly different between groups, which agreed with a previous study that observed mice treated for an acute toxicity period (Katisart and Konsue, 2019).



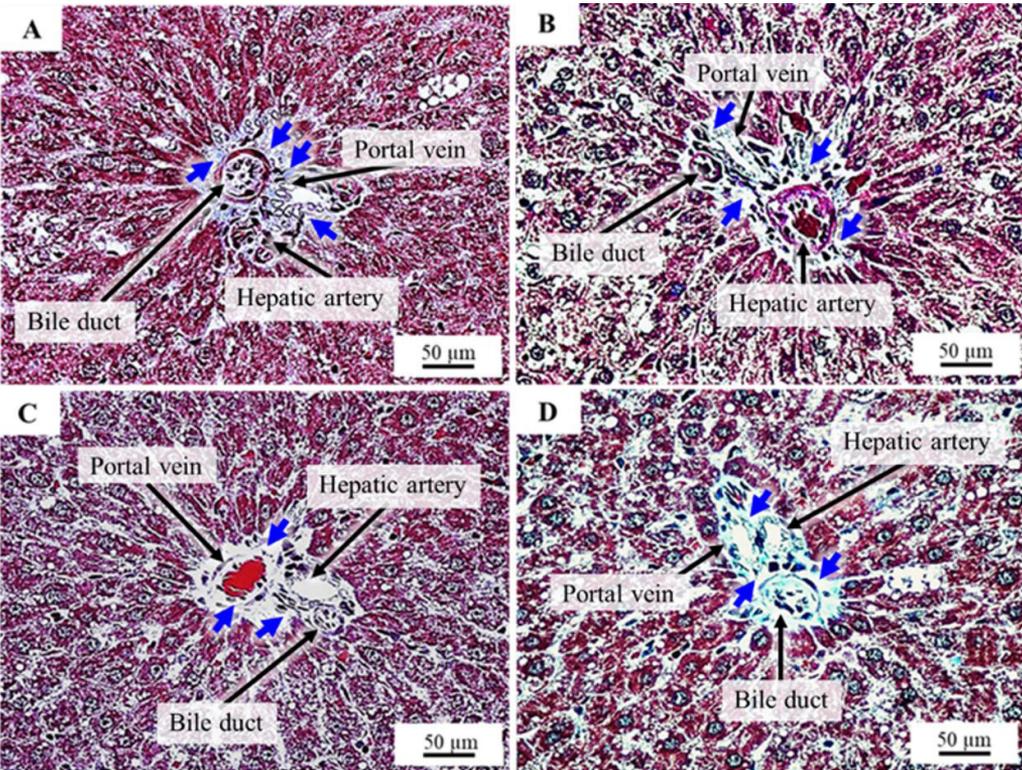
**Figure 1.** Representative histology of testis stained by Masson's trichrome of control (A), DSF100 (B), DSF300 (C), and DSF600 (D) groups, respectively. Red arrows = collagen fibers; SG = spermatogonia; RS = round spermatid.



**Figure 2.** Representative histology of caudal epididymis stained by Masson's trichrome of control (A), DSF100 (B), DSF300 (C), and DSF600 (D) groups, respectively. Black stars = collagen fibers.



**Figure 3.** Representative histology of kidney stained by Masson's trichrome of control (A), DSF100 (B), DSF300 (C), and DSF600 (D) groups, respectively. Blue arrows = collagen fibers; RT = renal tubules.



**Figure 4.** Representative histology of liver showing portal triad stained by Masson's trichrome of control (A), DSF100 (B), DSF300 (C), and DSF600 (D) groups, respectively. Blue arrows = collagen fibers.

Liver and kidney microstructures showed no obvious alterations after DSF administration at any dose. However, their functions were slightly altered due to decreased ALT and ALP levels, while the creatinine levels were significantly increased. Such functional changes could be explained by the activity of saponin found in DSF crude extract as described in a previous study (Kiratipai boon et al., 2012). This mechanism may be similar to that observed in treatment with sun ginseng, whose saponin content led to alterations of blood serum parameters tied to liver and kidney function such as ALT, ALP, and creatinine levels (Kim et al., 2013). However, such physiological alterations were not sufficient to severely damage liver and kidney structures. As shown in Table 2; biochemical parameters in blood serum from DSF treated groups were altered when compared to those of controls, however their levels were still within normal ranges including ALT [18–45 U/L], ALP [62–230 U/L], and creatinine [0.2–0.5 mg/dL] as previously reported in an animal model (Hayakawa et al., 2013; Pereira et al., 2017). As a study limitation, other toxic substances in DSF and apoptotic markers in tissues need to be further investigated to determine their safety for use and consumption over extended periods.

## 5. Conclusion

This study demonstrated that aqueous DSF flower extract had no toxicity on the vital organs and male reproductive tissues, but produced mild alterations of some parameters for liver and kidney function.

## 6. Future Prospects

Based on the findings of this article, the safety of DSF consumption over extended periods for renal and liver microstructures was demonstrated. Specifically, DSF extract is shown to be nontoxic for testis and epididymis. Hopefully, DSF extract can be further developed as a dietary supplement for male reproductive health.

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