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Subchronic toxicity test of *Strychnos ligustrina* extract and dihydroartemisinin-piperaquine phosphate in male and female mice

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ABSTRACT

Strychnos ligustrina extract and dihydroartemisinin-piperaquine phosphate (SL+DHP) may increase antimalarial potential. However, no research reported the toxic effect of SL+DHP. Therefore, this study investigated the subchronic toxicity of SL+DHP in male and female mice for 28 days. Subchronic toxicity tests were using National Agency of Drug and Food Control test guidelines. Mortality, bodyweight, and relative organ weight were measured. Blood samples were analyzed for hematological and biochemical parameters. Organs were examined for histopathological analysis. The highest mortality in mice was because of high doses (800 + 333 mg/kg BW) in male and female mice. The high dose significantly decreased body weight for 28-day treatments but increased after stopping administration for 2 weeks. The relative organ weight showed a significant change in the kidney, brain, and gonad of treatment groups, but its change was a recovery in satellite groups. *Strychnos ligustrina extract*+DHP in the high dose significantly changed the hematological and biochemical parameters of treatment groups, but these changes recovered in male and female mice of satellite groups. Histopathological examination revealed that *S. ligustrina extract*+DHP had a strong toxic effect in the kidney and caused ulcer compared to other organs. Subchronic toxicity of SL+DHP for 28 days was safe in low doses (200 + 111 mg/kg BW). Medium doses (400 + 222 mg/kg BW) and high doses (800 + 333 mg/kg BW) showed a toxic effect but recovered after stopping administration for 2 weeks.

INTRODUCTION

Strychnos ligustrina Blume commonly known as bidara laut in Indonesia is a deciduous tree and belongs to Loganiaceae family (Rahayu *et al*., 2022; Syafii *et al*., 2016). The plants taste bitter in all parts of the plant, especially the roots. In Indonesian people, this plant is commonly used as a traditional medicine: its bark for toothache and burns, root for stomach aches, and seeds and wood for antimalarial effect (Setiawan *et al*., 2014). Virtual screening found that bioactive compounds, like chlorogenic acid, phyllamycin A, strychnine, and brucine N-oxide, were predicted to have antidiabetic agents (Setiawansyah *et al*., 2022).

Phytochemical analysis showed that *S. ligustrina* wood extract was detected to contain alkaloids, phenol, hydroquinone, tannins, flavonoids, saponins, steroids, and terpenes (Manurung *et al*., 2019; Setiawan *et al*., 2014). The wood of *S. ligustrina* Blume syn. *S. lucida* R. Br. in East Nusa Tenggara (NTT), Indonesia, was traditionally used to treat people with malaria (Setiawan *et al*., 2014; Syafii *et al*., 2016). Syafii *et al*. (2016) successfully identified that two fractions contained from ethanol extract of *S. ligustrina* wood were noted as an antimalarial activity with IC_{50} values of 1.99 (F3) and 0.39 (F4) µg ml^{-1}. The bioactive profiling compound using

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gas chromatography-mass spectrometry revealed the presence of alkaloids, 4-methyl-5-[3-trifluoromethylphenoxy]-6-methoxy-8-nitroquinoline, and phenolic compounds in F3 fraction and furan and phenolic compounds, fatty acid, and alkaloid in F4 fraction.

Malaria is a disease caused by the protozoan, *Plasmodium* sp., with the female Anopheles mosquito as a vector. The World Health Organization (WHO) (2015) stated that future antimalarial medicine should be treated with at least two effective medicines with different mechanisms of action to prevent and reduce drug resistance. Furthermore, WHO recommended artemisinin-based combination therapy (ACT) to treat malaria. One of the ACTs commonly used is dihydroartemisinin-piperaquine phosphate (DHP). *Artocarpus champeden* Spreng. extract was used in combination with DHP to increase the effectiveness as an antimalarial treatment and possibly slow down the parasite resistance to the drug (Hafid *et al*., 2011). Until now, there have been no reports of subchronic toxicity of the combination of *S. ligustrina* extract and DHP. Therefore, this study aimed to investigate the subchronic toxicity of *S. ligustrina* extract and DHP in mice.

MATERIAL AND METHODS

Extraction and preparation

Strychnos ligustrina wood was harvested from West Nusa Tenggara (NTB), Indonesia, at April, 2021. A total of 30 logs (1 m) were cut from 15 trees. The wood of logs was cut and dried under sunlight. Then, the wood was shaved and ground to get powder. The powder was macerated in distilled water (1:8, *b*/*v*) for 24 hours. The maceration was conducted three times in the same powder. The filtrate was performed in a vacuum evaporator to get the dry extract and then was put in a capsule using a semiauto capsule filler. The average weight of the extract was 459.6 ± 24.56 mg.

The sample test was the combination of *S. ligustrina* extract and DHP-Frimal®. The basis for combinations was determined based on the effectivity test in the antimalarial inhibition test, which showed that the best ratio was 1 (*S. ligustrina* extract 200 mg/Kg BW): 0.5 (DHP 111 mg/kg BW) (Cahyaningsih *et al*., 2022). The tests were divided into three doses (*S. ligustrina* extract+DHP): the low dose was the effective dose (200 + 111 mg/kg BW); the medium dose was a 2-fold effective dose (400 + 222 mg/kg BW); the high dose was a 4-fold effective dose (800 + 333 mg/kg BW).

Animals

Adult albino mice (DDY strain; 6–8 weeks) were used for the subchronic studies and were obtained from the National Agency of Drug and Food Control (NA-DFC), the Republic of Indonesia. A total of 150 mice were 75 males and 75 females. Males were separated from females, and every cage was housed with five mice. All mice were acclimatized for 14 days in controlled conditions: 12-hours light/12-hours dark cycle, temperature 24°C ± 1°C, and humidity 55%–63%. The fed mice were given 5 g/mice, and they were free to access water (*ad libitum*). All procedures in the experiment were approved by the Animal Ethics Committee, IPB University (approval number: 154-2019).

Subchronic toxicity study

The assay of the subchronic study was performed according to the NA-DFC, Republic of Indonesia (BPOM, 2014). Each male and female mice were divided into the following:

Treatment groups (n = 10):

a. Group B: baseline (n = 5)
b. Group I: control (AQUADEST)
c. Group II: low dose (*S. ligustrina* 200 + DHP 111 mg/kg BW)
d. Group III: medium dose (*S. ligustrina* 400 + DHP 222 mg/kg BW)
e. Group IV: high dose (*S. ligustrina* 800 + DHP 333 mg/kg BW)

Satellite groups (n = 10):

a. Group V: low dose (*S. ligustrina* 200 + DHP 111 mg/kg BW)
b. Group VI: medium dose (*S. ligustrina* 400 + DHP 222 mg/kg BW)
c. Group VII: high dose (*S. ligustrina* 800 + DHP 333 mg/kg BW)

The satellite groups were used to evaluate the recovery process from toxic effects. The subchronic toxicity test was performed for 28 days. The *S. ligustrina* extract + DHP was administrated orally. The dead mice during the treatment were recorded. The bodyweight was weighed on days 0, 1, 7, 14, 21, and 28.

Blood and organ collection

Blood collection was conducted from group B at 0 days as a normal parameter. At 29 days, the treatment groups (I, II, and III) were anesthetized with ketamine-xylazine (70 mg/kg to 20 mg/kg BW), and blood (1 ml) was collected via intracardiac, followed by a secondary means of euthanasia, exsanguination. The blood was stored in a vacutainer with a heparin anticoagulant. After death, mice were sacrificed, and their organs were isolated to evaluate relative organ weights, namely liver, lung, kidney, brain, heart, lymph, ulcer, testis, ovarium, and uterus. The satellite groups (I, V, VI, and VII) were sacrificed 14 days after treatment.

Hematological and biochemistry study

Hematological analysis was performed using a hematology analyzer (Mindray BC-2800vet) to evaluate erythrocyte (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), MCH concentration (MCHC), red-cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), procalcitonin (PCT), leukocyte (WBC), lymphocyte, monocyte, and granulocyte (Zhang *et al*., 2021). Biochemistry analysis was performed using a clinical chemistry analyzer (SPOTCHEM™ EZ SP-4430) with the following parameters: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), total cholesterol total, glucose, urea (BUN), and creatinine (Lee *et al*., 2021). Blood electrolytes were evaluated using SPOTCHEM™ EL SE-1520 to calculate sodium (Na), potassium (K), and chlorine (Cl) (Okamoto *et al*., 2021).

Histopathology study

The liver, lung, kidney, brain, heart, lymph, ulcer, testis, ovarium, and uterus were fixed in buffer neutral formalin 10% for 7 days and were stored at the stopping point (ethanol 70%). Then, this tissue was trimmed and dehydrated in a graded ethanol series (80%, 90%, 95%, and 100%). The clearing of tissues was carried out using xylene, paraffin infiltration, and paraffin embedding. The embedded tissues were cut using a rotary microtome at a thickness of 5 µm. The sections were stained with hematoxylin-eosin and observed under a light microscope (400×). The analysis was examined in the following parameters: degeneration, necrosis, necrosis/degeneration, inflammation, regeneration, pneumonia, bronchitis, peribronchitis, and edema. The score was 0: no damage, 1: <25% damage, 2: ≥25%–50% damage, 3: ≥50%–75% damage, and 4: ≥75% damage.

Statistical analysis

The data were analyzed using one-way ANOVA followed by Duncan's multiple comparison test (Duncan's test). If the data were not in normal distribution and homogeneity after transformation, Kruskal–Wallis test followed by the Mann–Whitney test was used for analysis.

RESULT

Mortality

The ratio of animals with mortality after *S. ligustrina* extract+DHP treatments was shown in Table 1. In the male groups, the total ratio of dead/treated animals for 5 weeks from the highest to the lowest was high dose (11/20), low dose (5/20), medium dose (3/20), and control groups (1/10). The mortality in male groups was unrelated to the dose. In female groups, high-dose groups (12/10) exhibited the highest ratio of animal mortality, followed by medium-dose groups (5/20). In contrast, control and low-dose groups (2/20) had the same mortality ratio in animals.

Bodyweight and relative organ

Data related to relative organ weight in male and female mice treated with *S. ligustrina* extract+DHP were shown in Tables 2 and 3. The body weight in high-dose treatment groups significantly differed from control on the 7th–28th day for male mice (Table 2). In female mice of treatment groups, high doses showed a significant increase in body weight from the 21st to 28th compared to control, low, and medium doses. The body weight in the high-dose satellite groups was not significantly different from the 35th to the 42nd. The research showed that *S. ligustrina extract*+DHP in high dose significantly decreased bodyweight, but these effects were not permanent (reversible), as indicated by increasing bodyweight in satellite groups.

The relative organ weight of control and treatment groups, including liver, lymph, ulcer, lung, and heart, did not reveal significant change after *S. ligustrina* extract+DHP administration (Table 3). A significant change in the relative weight of the kidney was found in male and female treatment groups, but it was a non-dose-dependent increase. The relative organ weight of the kidney (1.54% ± 0.11%) in high dose increased significantly compared to in control (1.22% ± 0.11%) and low dose (1.17 ± 0.08). Brain and gonad in female mice of treatment groups showed a significant change in relative organ weight, but not in male mice of treatment groups. The relative organ weight of the brain in control was not different from in other doses. However, a high dose (0.42 ± 0.30) showed a decrease in the relative weight of gonad compared to the control (1.33 ± 0.47). There was no significant change in relative organ weight in all organs observed in satellite groups. The study showed that the administration of *S. ligustrina* extract+DHP significantly affected the relative weight of kidneys and gonads of mice, but their effects were reversible.

Hematological analyses

The results of hematological parameter analysis in male and female mice were presented in Tables 4 and 5, respectively. No significant change among male mice in treatment and satellite groups was noted in the level of Hb, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, WBC, lymphocyte, monocyte, and granulocyte. The level of erythrocyte (RBC) in male mice (treatment and satellite groups) showed a significant change. The level of RBC in the high dose (11.64 ± 0.056 × 10^6/µl) of the

Table 1. The mortality of mice after the combination of *S. ligustrina* extract and DHP treatments.

Groups*	Died animals/treated animals					Total died/treated animals
	Week 1	Week 2	Week 3	Week 4	Week 5	
Male						
Control	0/20	0/20	1/20	0/20	0/20	1/20
Low dose	0/20	020	4/20	1/20	0/20	5/20
Medium dose	1/20	0/20	1/20	0/20	1/20	3/20
High dose	3/20	4/20	4/20	0/20	0/20	11/20
Female						
Control	1/20	1/20	0/20	0/20	0/20	2/20
Low dose	0/20	1/20	1/20	0/20	0/20	2/20
Medium dose	1/20	2/20	1/20	0/20	1/20	5/20
High dose	2/20	9/20	1/20	0/20	0/20	12/20

*Groups consisted of treatment and satellite groups. Low dose: 200 + 111 mg/kg BW. Medium dose: 400 + 222 mg/kg BW. High dose: 800 + 333 mg/kg.

Table 2. Effect of combination of *S. ligustrina* extract and DHP on body weights in mice.

Days	Treatment groups (g)				Satellite groups (g)			
	Control	Low dose	Medium dose	High dose	Control	Low dose	Medium dose	High dose
Males								
0	33.33 ± 2.16	34.33 ± 2.42	33.50 ± 0.83	33.67 ± 2.10	34.33 ± 4.50	30.67 ± 2.08	35.00 ± 2.64	33.00 ± 4.35
1	32.50 ± 2.07	31.50 ± 3.20	32.50 ± 3.08	30.67 ± 3.66	33.33 ± 3.51	30.33 ± 1.52	31.00 ± 3.06	30.33 ± 2.88
7	34.50 ± 3.88b	33.00 ± 2.75b	33.16 ± 1.47b	29.33 ± 3.14a	32.67 ± 2.81	30.33 ± 4.04	29.33 ± 4.72	30.00 ± 2.64
14	32.16 ± 5.45b	31.80 ± 3.33b	34.67 ± 3.50b	24.33 ± 4.76a	34.00 ± 6.08	30.33 ± 4.93	27.33 ± 3.78	25.67 ± 5.49
21	36.50 ± 3.50c	31.83 ± 3.06b	32.16 ± 2.13b	24.33 ± 4.27a	35.33 ± 5.50	30.67 ± 5.50	25.67 ± 3.21	25.00 ± 6.24
28	34.67 ± 2.42c	31.67 ± 3.38bc	31.16 ± 1.83b	22.67 ± 2.33a	37.00 ± 1.00c	30.66 ± 3.51b	27.33 ± 4.72b	21.00 ± 1.00a
35	-	-	-	-	36.00 ± 5.19	30.67 ± 2.30	28.00 ± 3.60	27.73 ± 4.96
42	-	-	-	-	33.67 ± 5.13	27.67 ± 2.30	33.33±1.52	28.93 ± 3.40
Females								
0	31.33 ± 1.58	31.33 ± 1.15	31.33 ± 2.51	33.33 ± 1.15	29.00 ± 2.64	30.00 ± 1.00	32.00 ± 2.00	33.00 ± 2.00
1	32.00 ± 3.00	28.66 ± 1.52	31.33 ± 3.05	29.67 ± 4.72	27.67 ± 5.85	30.33 ± 1.52	31.00 ± 1.00	33.33 ± 1.52
7	31.33 ± 1.52	30.33 ± 1.52	33.33 ± 2.30	28.00 ± 5.56	29.67 ± 2.08	29.00 ± 1.00	31.00 ± 1.00	31.67 ± 2.30
14	31.00 ± 1.00	28.66 ± 2.51	31.33 ± 2.30	24.67 ± 7.37	27.67 ± 2.08	28.86 ± 0.57	30.00 ± 2.00	29.33 ± 3.05
21	32.66 ± 1.15b	29.67 ± 1.52b	31.00 ± 2.00a	24.33 ± 4.04a	33.00 ± 1.00	29.67 ± 1.15	31.00 ± 1.73	29.33 ± 3.05
28	32.67 ± 0.57ab	30.33 ± 0.57b	33.33 ± 1.15a	24.67 ± 2.89c	32.00 ± 1.00	30.00 ± 2.64	29.67 ± 3.51	28.00 ± 2.64
35	-	-	-	-	31.33 ± 1.15	29.67 ± 1.52	30.33 ± 1.15	27.33 ± 2.30
42	-	-	-	-	34.67 ± 1.52	32.00 ± 1.00	33.00 ± 0.00	30.67 ± 2.51

Low dose: 200 + 111 mg/kg BW. Medium dose: 400 + 222 mg/kg BW. High dose: 800 + 333 mg/kg BW. Values are expressed as means ± SD (n = 3). Numbers followed by the same letters (a–c) in the same row were not significantly different from Duncan's test results (α = 0.05).

Table 3. Effect of combination of *S. ligustrina* extract and DHP on the relative organ weights in mice.

Groups		Parameters (%)							
		Liver	Kidney	Lymph	Ulcer	Lung	Heart	Brain	Gonad
Treatment groups									
♂	Control	5.98 ± 0.34	1.41 ± 0.23a	0.30 ± 0.07	1.68 ± 0.28	0.98 ± 0.17	0.46 ± 0.30	1.21 ± 0.21	0.61 ± 0.03
	Low dose	6.98 ± 0.33	1.70 ± 0.04ab	0.52 ± 0.10	1.80 ± 0.11	0.97 ± 0.39	0.52 ± 0.27	1.35 ± 0.29	1.03 ± 0.37
	Medium dose	5.27 ± 0.63	1.49 ± 0.06a	0.47 ± 0.24	1.34 ± 0.23	0.79 ± 0.16	0.38 ± 0.02	0.92 ± 0.30	1.02 ± 0.34
	High dose	6.25 ± 1.41	1.99 ± 0.28b	0.60 ± 0.03	1.42 ± 0.11	1.10 ± 0.26	0.47 ± 0.18	1.27 ± 0.079	1.10 ± 0.22
♀	Control	5.88 ± 1.08	1.22 ± 0.11a	0.48 ± 0.22	1.77 ± 0.28	0.84 ± 0.19	0.41 ± 0.049	1.24 ± 0.16ab	1.33 ± 0.47b
	Low dose	5.40 ± 0.31	1.17 ± 0.08a	0.42 ± 0.10	1.85 ± 0.22	0.84 ± 0.14	0.40 ± 0.22	1.35 ± 0.09b	0.83 ± 0.26ab
	Medium dose	5.98 ± 0.56	1.42 ± 0.18ab	0.48 ± 0.11	1.95 ± 0.32	0.97 ± 0.25	0.46 ± 0.09	1.03 ± 0.11a	0.81 ± 0.13ab
	High dose	6.68 ± 0.31	1.54 ± 0.11b	0.45 ± 0.19	1.67 ± 0.40	0.98 ± 0.19	0.55 ± 0.21	1.39 ± 0.09b	0.42 ± 0.30a
Satellite groups									
♂	Control	4.58 ± 0.85	1.34 ± 0.18	0.45 ± 0.05	1.34 ± 0.18	0.50 ± 0.08	0.43 ± 0.02	1.23 ± 0.11	1.31 ± 0.59
	Low dose	4.95 ± 0.57	1.25 ± 0.34	0.45 ± 0.15	1.39 ± 0.35	0.65 ± 0.057	0.40 ± 0.01	1.14 ± 0.23	0.099 ± 0.14
	Medium dose	6.35 ± 1.16	1.33 ± 0.46	0.60 ± 0.24	1.42 ± 0.35	0.66 ± 0.25	0.41 ± 0.13	1.25 ± 0.33	1.30 ± 0.66
	High dose[#]	6.08	1.76	0.40	1.84	1.04	0.40	1.20	0.9
♀	Control	5.02 ± 0.18	1.01 ± 0.08	0.55 ± 0.20	0.98 ± 0.21	0.72 ± 0.16	0.32 ± 0.06	0.93 ± 0.08	0.64 ± 0.19
	Low dose	5.38 ± 0.56	1.13 ± 0.17	0.68 ± 0.24	1.16 ± 0.17	1.72 ± 1.91	1.60 ± 2.12	1.06 ± 0.05	0.81 ± 0.35
	Medium dose	3.89 ± 2.14	1.12 ± 0.07	0.40 ± 0.01	1.23 ± 0.21	0.6 ± 0.12	0.39 ± 0.10	1.04 ± 0.10	0.65 ± 0.33
	High dose	5.77 ± 0.72	1.43 ± 0.27	0.60 ± 0.16	1.25 ± 0.19	0.61 ± 0.16	0.31 ± 0.06	0.98 ± 0.025	0.58 ± 0.08

Low dose: 200 + 111 mg/kg BW. Medium dose: 400 + 222 mg/kg BW. High dose: 800 + 333 mg/kg BW. Values are expressed as means ± SD (n = 3). ([#]): n = 1, one mouse at the end of treatment. Numbers followed by the same letters (a–c) in the same column were not significantly different from Duncan's test results (α = 0.05).

Table 4. Effect of combination of *S. ligustrina* extract and DHP on hematological parameters in male mice.

Parameters	Treatment groups				Satellite groups				Unit	Normal range
	Control	Low dose	Medium dose	High dose	Control	Low dose	Medium dose	High dose		
Erythrocyte (RBC)	8.67 ± 0.15a	8.90 ± 0.61a	9.44 ± 1.85a	11.64 ± 0.056b	8.71 ± 0.72ab	9.87 ± 0.43b	8.02 ± 0.83a	8.4	10^6 /µl	5.3–10 10^6/µl
Hb	12.87 ± 0.50	12.55 ± 0.44	14.17 ± 3.39	17.00 ± 0.361	13.03 ± 1.20	13.90 ± 1.31	11.47 ± 1.00	11.8	g/dl	14–18 g/dl
HCT	40.27 ± 1.06	38.40 ± 0.92	41.20 ± 10.75	51.53 ± 1.686	41.37 ± 2.78ab	44.87 ± 3.63b	36.73 ± 2.86a	37.1	%	36%–46%
MCV	46.53 ± 0.64	43.60 ± 1.93	44.70 ± 0.95	44.30 ± 1.217	47.63 ± 2.02	45.53 ± 3.82	44.60 ± 1.61	44.2	fl	53–68.8 fl
MCH	14.80 ± 0.36	14.05 ± 0.56	14.87 ± 0.72	14.57 ± 0.289	14.90 ± 0.80	14.07 ± 1.36	14.23 ± 0.35	14	Pg	16–23.1 pg
MCHC	31.90 ± 0.40	32.65 ± 0.57	33.37 ± 0.83	32.93 ± 0.833	31.43 ± 0.81	30.90 ± 0.46	31.13 ± 0.31	31.8	g/dl	30–34.1 g/d
RDW	13.77 ± 0.97	13.10 ± 1.50	14.47 ± 0.45	14.00 ± 0.346	15.90 ± 1.01	15.30 ± 0.56	16.30 ± 1.25	14.1	%	11.0%–15.5%
PLT	1,147.00 ± 93.15	570.00 ± 424.50	627.00 ± 363.87	832.33 ± 361.57	961.00 ± 388.95	1,246.67 ± 394.06	629.67 ± 387.77	1043	10^3/µl	100–1,610 10^3 /µl
MPV	4.30 ± 0.26	4.20 ± 0.20	4.73 ± 1.17	4.73 ± 0.231	5.17 ± 1.15	4.87 ± 0.51	4.63 ± 0.06	4	%	%
PDW	16.03 ± 0.12	16.10 ± 0.23	16.20 ± 0.56	16.33 ± 0.231	16.60 ± 0.90	16.67 ± 0.65	16.33 ± 0.15	15.8	fl	3.8–6.2 10^3 fl
PCT	0.49 ± 0.02	0.33 ± 0.11	0.27 ± 0.13	0.39 ± 0.156	474.33 ± 165.54	510.50 ± 122.33	289.67 ± 176.98	417	%	%
WBC	4.50 ± 1.35	4.80 ± 1.60	9.50 ± 7.37	7.67 ± 0.473	5.43 ± 1.85	6.70 ± 3.68	5.07 ± 0.80	7.5	10^3/µl	2.9–15.3 10^3/ µl
Lymphocyte	62.73 ± 2.55	59.15 ± 3.72	52.93 ± 11.72*	38.90 ± 0.954*	62.97 ± 10.95	54.03 ± 27.66	67.17 ± 3.23	51.2	%	63.7%–90.1%
Monocyte	3.65 ± 0.49	3.63 ± 0.38	5.43 ± 1.15	3.63 ± 1.012	4.53 ± 2.17	5.93 ± 3.33	3.80 ± 0.87	5.3	%	1.5%–4.5%
Granulocyte	33.60 ± 2.78	39.17 ± 3.45	41.63 ± 10.72	57.47 ± 1.361*	32.50 ± 9.25	40.03 ± 25.23	29.03 ± 2.42	43.5	%	7.3%–30.1%

MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red-cell distribution width, MPV: mean platelet volume, PDW: platelet distribution width, PCT: procalcitonin. Low dose: 200 + 111 mg/kg BW. Medium dose: 400 + 222 mg/kg BW. High dose: 800 + 333 mg/kg BW. Values are expressed as means ± SD ($n = 3$). Numbers followed by the same letters (a–c) in the same row were not significantly different from Duncan's test results ($\alpha = 0.05$). *$p < 0.05$ by Mann–Whitney test compared to control in one row.

Table 5. Effect of combination of *S. ligustrina* extract and DHP on hematological parameters in female mice.

Parameters	Treatment groups				Satellite groups				Unit	Normal range
	Control	Low dose	Medium dose	High dose	Control	Low dose	Medium dose	High dose		
Erythrocyte (RBC)	8.47 ± 0.53	7.84 ± 0.34	8.29 ± 1.16	8.74 ± 0.77	8.4 ± 0.4ab	8.81 ± 0.31b	8.40 ± 0.17ab	7.91 ± 0.30a	10^6/µl	5.3–10 10^6 /µl
Hb	13.17 ± 0.87	11.97 ± 0.31	12.33 ± 1.50	12.53 ± 1.34	12.5 ± 0.8a	13.83 ± 0.85b	12.57 ± 0.47a	11.30 ± 0.46a	g/dl	14–18 g/dl
HCT	41.30 ± 3.58	37.67 ± 1.36	39.20 ± 5.02	38.53 ± 3.23	39.3 ± 2.1bc	42.63 ± 2.54c	38.70 ± 1.39b	35.03 ± 1.33a	%	36%–46%
MCV	48.73 ± 1.27b	47.75 ± 0.35b	47.43 ± 2.31b	44.13 ± 0.29a	47.0 ± 1.7	48.40 ± 1.22	46.13 ±1.10	44.40 ± 1.73	fl	53–68.8 fl
MCH	15.50 ± 0.10b	15.20 ± 0.35b	14.83 ± 0.75ab	14.27 ± 0.35a	14.9 ± 0.6ab	15.63 ± 0.45c	14.90 ± 0.36ab	14.23 ± 0.35a	Pg	16–23.1 pg
MCHC	31.90 ± 0.70	31.73 ± 0.35	31.43 ± 0.21	32.43 ± 0.91	31.9 ± 0.3	32.37 ± 0.31	32.43 ± 0.31	32.20 ± 0.53	g/dl	30–34.1 g/dl
RDW	13.63 ± 1.70	14.77 ± 0.23	14.97 ± 0.55	13.00 ± 1.14	14.9 ± 0.2	15.13 ± 0.81	15.40 ± 1.44	14.47 ± 0.75	%	11.0%–15.5%
PLT	939.00 ± 61.80	966.67 ± 216.25	944.33 ± 153.02	789.33 ± 284.95	773.0 ± 650.5	1,023.67 ± 317.41	1,107.33 ± 233.82	1,033.33 ± 4.93	10^3/µl	100–1,610 10^3/µl
MPV	4.63 ± 0.32	5.00 ± 0.56	4.97 ± 0.68	4.33 ± 0.35	5.0 ± 0.6	4.43 ± 0.29	4.20 ± 0.17	4.33 ± 0.29	%	%
PDW	16.00 ± 0.10	16.40 ± 0.46	16.23 ± 0.57	15.93 ± 0.21	17.1 ± 0.9b	16.33 ± 0.29ab	15.83 ± 0.12a	15.97 ± 0.25a	fl	3.8–6.2 10^3 fl
PCT	435.67 ± 57.74	484.67 ± 124.99	462.33 ± 43.13	335.33 ± 94.87	355.7 ± 297.6	451.00 ± 136.52	466.67 ± 112.54	447.33 ± 30.66	%	%
WBC	4.37 ± 3.67	8.37 ± 4.94	4.33 ± 1.38	5.40 ± 2.08	5.1 ± 2.5	5.17 ± 0.75	7.87 ± 1.76	5.47 ± 2.59	10^3/µl	2.9–15.3 10^3/µl
Lymphocyte	65.07 ± 7.51	68.83 ± 9.75	64.00 ± 21.22	45.70 ± 5.34	66.4 ± 22.2	75.90 ± 2.54	70.07 ± 6.49	62.20 ± 6.58	%	63.7%–90.1%
Monocyte	4.73 ± 1.62	3.83 ± 1.08	3.30 ± 1.39	4.60 ± 1.65	4.8 ± 4.0	2.80 ± 0.17	3.37 ± 0.15	5.20 ± 1.76	%	1.5%–4.5%
Granulocyte	30.20 ± 6.32b	27.33 ± 8.75b	8.70 ± 12.47a	49.70 ± 4.37c	28.8 ± 18.3	21.30 ± 2.43	26.57 ± 6.36	32.60 ± 5.28	%	7.3%–30.1%

MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red-cell distribution width, MPV: mean platelet volume, PDW: platelet distribution width, PCT: procalcitonin. Low dose: 200 + 111 mg/kg BW. Medium dose: 400 + 222 mg/kg BW. High dose: 800 + 333 mg/kg BW. Values are expressed as means ± SD ($n = 3$). Numbers followed by the same letters (a–c) in the same row were not significantly different from Duncan's test results ($\alpha = 0.05$).

treatment group increased significantly compared to the control (8.67 ± 0.15 × 10^6/µl). HCT level in satellite groups showed a significant change but not in treatment groups. However, RBC and HCT in medium (8.02 ± 0.83 × 10^6/µl; 36.73% ± 2.86%) and low dose (9.87 ± 0.43 × 10^6/µl; 44.87% ± 3.63%, respectively) were not significantly different in comparison with control (8.71 ± 0.72 × 10^6/µl; 41.37% ± 2.78%) in satellite groups. These changes were not dose related. In the treatment groups, the level of lymphocyte and granulocyte in medium and high doses (52.93% ± 11.7% and 41.63% ± 10.72%; 38.90% ± 0.95% and 57.47% ± 1.36%, respectively) was significantly different from the control (62.73% ± 2.55% and 33.60% ± 2.78%, respectively), but not for granulocyte in medium dose (41.63% ± 10.72%).

The hematological analysis in female treatment groups revealed a statistical change in some parameters, i.e., MCV, MCH, and granulocyte 2, while other parameters showed no significant change (Table 5). The value of MCV and MCH in high dose of treatment groups (44.13 ± 0.29 fl and 14.27 ± 0.35 Pg) decreased significantly compared to control (48.73 ± 1.27 fl and 15.50 ± 0.10 Pg). In addition, the high dose (49.70% ± 4.37%) in treatment groups showed a significant increase in granulocyte compared to the control (30.20% ± 6.32%). A significant change in the level of RBC, Hb, HCT, MCH, and PDW was noted in female satellite groups treated with three-level doses compared to the control groups. However, these changes were not dose related. The level of HCT and PDW in the high dose of satellite groups (35.03% ± 1.33% and 15.97 ± 0.25fl) decreased significantly compared to the control (39.3% ± 2.1% and 17.1 ± 0.9fl). The result showed that *S. ligustrina extract*+DHP in the high dose significantly changed the hematological parameters of treatment groups, but these changes recovered in male and female mice of satellite groups. The best recovery was in hematological parameters found in the male mice.

Biochemical analyses

The effect of subchronic toxicity of *S. ligustrina* extract+DHP on biochemical parameters was shown in Tables 6 and 7. The treatment groups in male mice showed a significant increase in ALT, AST, and creatinine after increasing the dose level (Table 6). The level of ALT and AST in the high dose of treatment groups (275.00 ± 90.510 IU/l and 411.00 ± 104.652 IU/l) increased significantly compared to the control (26.50 ± 2.12 and 76.50 ± 20.51, respectively). Likewise, high and medium doses of treatment groups (0.43±0.404 mg/dl and 0.20 ± 0.10 mg/dl) showed a significant increase in creatinine levels compared to the control (0.07 ± 0.06 mg/dl). On the other hand, the satellite group in male mice only exhibited a significant change in Na^+ ion.

In female mice of treatment groups, a significant increase in ALT and AST levels was noted in the high dose (147 ± 21.21 IU/l and 240.5 ± 41.72 IU/l) compared to the control (78.50 ± 55.86 IU/l and 77.00 ± 28.28 IU/l, respectively). Also, the satellite groups in female mice showed a significant change in ALT and glucose levels. The level of ALT in the high dose (84.33 ± 12.74 IU/l) of the satellite group was significantly different from the control (24.7 ± 23.5 IU/l). The result showed that *S. ligustrina* extract+DHP in the high-dose treatment groups significantly changed biochemical parameters (ALT and AST), but

Table 6. The effect of the combination of *S. ligustrina* extract and DHP on biochemical parameters in male mice.

Parameters	Treatment groups				Satellite groups			Unit	Normal range
	Control	Low dose	Medium dose	High dose	Control	Low dose	Medium dose	High dose		
ALP	84.5 ± 12.02	131.33 ± 76.42	122 ± 101.82	65 ± 34.073	80.50 ± 34.65	177.67 ± 161.11	127.33 ± 26.03	138	IU/l	86–174 IU/l
ALT	26.50 ± 2.12a	63.67 ± 31.34a	149.00 ± 130.11ab	275.00 ± 90.510b	48.00 ± 28.28	45.00 ± 23.43	101.33 ± 34.59	165	IU/l	10–118 IU/l
AST	76.50 ± 20.51a	88.00 ± 14.80a	148.50 ± 61.52ab	411.00 ± 104.652b	108.33 ± 32.81	105.00 ± 9.17	142.00 ± 60.56	219	IU/l	63–127 IU/l
Protein total (PT)	4.80 ± 0.00	5.20 ± 1.04	4.50 ± 0.99	4.23 ± 0.451	5.57 ± 1.52	5.63 ± 1.29	4.47 ± 0.23	4.7	g/dl	6.6–7.6 g/dl
Cholesterol total	112.50 ± 2.12	125.67 ± 28.01	100.50 ± 17.68	103.67 ± 9.074	114.33 ± 25.15	104.33 ± 15.18	102.00 ± 6.08	99	mg/dl	68–170 mg/dl
Glucose	157.50 ± 3.54	142.33 ± 61.33	111.50 ± 103.94	64.33 ± 23.714	87.33 ± 30.73	105.00 ± 59.76	93.33 ± 12.74	128	mg/dl	98–132 mg/dl
Urea (BUN)	25.50 ± 3.54	20.33 ± 4.16	26.50 ± 4.95	25.33 ± 1.528	24.67 ± 6.35	25.33 ± 13.05	19.67 ± 1.15	29	mg/dl	17–21 mg/dl
Creatinine	0.07 ± 0.06c	0.10 ± 0.00bc	0.20 ± 0.10ab	0.43 ± 0.404a	0.17 ± 0.12	0.17 ± 0.06	0.07 ± 0.06	0.1	mg/dl	0.6–0.8 mg/dl
Na	149.00 ± 1.00	148.33 ± 1.53	147.00 ± 2.65	152.00 ± 3.606	149.33 ± 2.52a	160.00 ± 3.46b	147.33 ± 4.16a	146	mmol/l	138–160 mmol/l
K	4.30 ± 0.57	4.87 ± 0.83	6.10 ± 1.01	6.77 ± 0.603	5.10 ± 0.87	4.83 ± 0.84	5.77 ± 1.75	5.4	mmol/l	3.7–5.8 mmol/l
Cl	119.33 ± 1.53	123.00 ± 1.73	118.33 ± 2.31	126.00 ± 6.557	125.33 ± 5.69	134.33 ± 4.73	125.33 ± 4.16	130	mmol/l	102–145 mmol/l

ALP: alkaline phosphatase, ALT: alanine aminotransferase, and AST: aspartate aminotransferase. Low dose: 200 + 111 mg/kg BW. Medium dose: 400 + 222 mg/kg BW. High dose: 680 + 333 mg/kg BW. Values are expressed as means ± SD ($n = 3$). Numbers followed by the same letters (a–c) in the same row were not significantly different from Duncan's test results ($\alpha = 0.05$).

these changes recovered in the satellite groups. The best recovery in biochemical parameters was found in the male mice.

Histopathological analyses

The vital organs at the end of treatments, including liver, lung, kidney, brain, heart, lymph, ulcer, testis, ovarium, and uterus, were evaluated with the histopathological examination (Table 8). In general, the liver (0–1.3), kidney (0–2.3), and ulcer (0–2) exhibited higher abnormalities such as necrosis, degeneration, and inflammation than other organs in all doses. The medium abnormality was found in the brain (0–1.67) and lung (0–2.33). Ovarium (0–1), heart (0–1.5), testis (0–0.5), and lymph (0–1.5) showed a low abnormality. No abnormality was noted in the uterus (0). Interestingly, no degeneration in the liver was observed in the satellite groups at all doses, but necrosis (0.67–1.33) and inflammation (0.67–1.00) in the liver increased at low and medium doses. Interestingly, regeneration cell in the liver was observed in medium (1.00) and high dose (1.33). In the kidney, the treatment groups exhibited necrosis/degeneration (0–1.3) and inflammation (0–1.3), while the satellite groups showed an increase in necrosis/degeneration (0–2.33) and inflammation (0–2.33). The abnormality of ulcers, brain, and lung in satellite groups increased compared to the treatment groups. In the ovarium (0–1.67) and lymph (0–1.67), the abnormality only was found in satellite groups. Based on the data, it can be concluded that *S. ligustrina* extract+DHP had a strong effect on the kidney and ulcer compared to other organs.

DISCUSSION

The combination of *S. ligustrina* and DHP may increase antimalarial potential. However, no toxicity study of this combination has been reported. This study was the first report about the subchronic study of *S. ligustrina*+DHP. All doses (low, medium, and high doses) induced mortality in mice but had a non-dose-dependent increase. Previous research reported that methanol extract of *S. ligustrina* had a toxic effect on Nile tilapia (*Oreochromis niloticus*), such as decreasing HCT and leucocyte levels (Zubaidah *et al*., 2018). The high mortality was shown at the high doses in male and female groups, while the lowest mortality was in control groups (Table 1). The mortality in control groups was affected by cannibalism in mice.

Bodyweight changes, as sensitivity indication, are commonly used as an indicator and marker of side effects in toxicological evaluation (Traesel *et al*., 2014). In this study, the high dose in satellite groups consistently decreased body weight from the 7th day to the 28th day in treatment groups and on the 28th day in male satellite groups (Table 2). Interestingly, the body weight increased after *S. ligustrina*+DHP administration was discontinued for 2 weeks in satellite groups. Relative organ weight is to examine pathological changes in disturbed organs (Nugroho *et al*., 2020). The significant relative organ weights were noted in the kidney (female and male mice), brain (female mice), and gonad (female mice) (Table 3). Interestingly, there was no significant change in the relative organ weights in satellite groups. The result showed that *S. ligustrina*+DHP was toxic in body weight and relative organ weight, but its effect was not permanent in mice.

Hematological and biochemical parameters were measured to evaluate the organ disorder. Nonetheless, the repeated administration of *S. ligustrina*+DHP for 28 days may

Table 7. The effect of the combination of *S. ligustrina* extract and DHP on biochemical parameters in female mice.

Parameters	Treatment groups				Satellite groups				Unit	Normal range
	Control	Low dose	Medium dose	High dose	Control	Low dose	Medium dose	High dose		
ALP	82.00 ± 11.31	94.33 ± 9.07	149.00 ± 86.27	72.50 ± 20.51	176.0 ± 97.9	247.67 ± 69.53	173.33 ± 93.24	116.33 ± 26.84	IU/l	86–174 IU/l
ALT	78.50 ± 55.86a	36.67 ± 9.71a	64.50 ± 9.19a	147 ± 21.21b	24.7 ± 23.5ab	18.33 ± 7.64a	48.67 ± 4.62b	84.33 ± 12.74c	IU/l	10–118 IU/l
AST	77.00 ± 28.28a	67.00 ± 3.46a	106.00 ± 35.36a	240.5 ± 41.72b	79.3 ± 45.4	57.67 ± 5.13	68.00 ± 18.36	98.67 ± 5.69	IU/l	63–127 IU/l
TP	5.05 ± 0.35	4.37 ± 0.40	4.65 ± 0.07	4.8 ± 0.71	5.4 ± 0.6	5.27 ± 0.40	4.97 ± 0.49	4.73 ± 0.50	g/dl	6.6–7.6 g/dl
Cholesterol total	73.00 ± 15.56	107.67 ± 5.51	100.50 ± 28.99	92.5 ± 0.71	88.7 ± 8.6	88.33 ± 6.03	102.00 ± 14.93	85.67 ± 10.69	mg/dl	68–170 mg/dl
Glucose	194.00 ± 43.84	214.33 ± 30.92	156.00 ± 19.80	115 ± 56.57	143.3 ± 14.2a	150.67 ± 5.86a	201.00 ± 11.53b	163.33 ± 31.56a	mg/dl	98–132 mg/dl
Urea (BUN)	25.00 ± 2.83	20.00 ± 1.73	23.50 ± 2.12	22.5 ± 0.71	22.0 ± 4.4	16.33 ± 1.53	21.33 ± 3.51	20.33 ± 2.52	mg/dl	17–21 mg/dl
Creatinine	0.16 ± 0.11	0.03 ± 0.06	0.10 ± 0.00	0.07 ± 0.06	0.2 ± 0.1	0.10 ± 0.00	0.13 ± 0.06	0.17 ± 0.06	mg/dl	0.6–0.8 mg/dl
Na	152.00 ± 4.36	149.00 ± 2.00	151.00 ± 2.00	155.33 ± 0.58	151.0 ± 1.0	148.00 ± 3.00	149.33 ± 0.58	147.67 ± 1.15	mmol/l	138–160 mmol/l
K	8.03 ± 6.00	4.40 ± 0.78	7.20 ± 1.90	4.7 ± 1.13	5.1 ± 0.5	4.90 ± 0.66	4.47 ± 0.15	4.70 ± 0.66	mmol/l	3.7–5.8 mmol/l
Cl	125.67 ± 2.52	123.67 ± 0.58	127.00 ± 4.36	126 ± 4.36	125.3 ± 5.8	127.33 ± 2.31	124.33 ± 0.58	119.67 ± 6.03	mmol/l	102–145 mmol/l

ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase. Low dose: 200 + 111 mg/kg BW. Medium dose: 400 + 222 mg/kg BW. High dose: 800 + 333 mg/kg BW. Values are expressed as means ± SD (n = 3). Numbers followed by the same letters (a–c) in the same row were not significantly different from Duncan's test results (α = 0.05).

Table 8. Histopathological examination of mice organ on subchronic toxicity test after the combination of *S. ligustrina* extract and DHP administration.

	Groups	Liver				Lung				Kidney		Brain		Heart		Lymph		Ulcer		Testis		Ovarium		Uterus	
		Deg	Nec	Inf	Reg	Pne	Bro	Pbro	Oed	ND	Inf	ND	Inf	ND	Inf	ND	Inf	ND	Inf	ND	Inf	ND	Inf	ND	Inf
Treatment groups																									
♂	Control	0.00	0.33	0.33	0.00	0.33	0.00	0.00	0.00	0.67	0.33	1.33	1.33	0.00	0.00	0.00	0.00	0.33	0.33	0.33	0.00	×	×	×	×
	Low dose	0.67	0.67	0.67	0.00	0.67	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.50	0.00	0.00	0.00	0.67	0.67	0.00	0.00	×	×	×	×
	Medium dose	0.00	0.33	0.33	0.00	1.67	0.67	0.00	0.00	1.50	1.00	0.33	0.33	0.00	0.00	0.00	0.00	0.33	0.33	0.00	0.00	×	×	×	×
	High dose	0.00	1.00	1.00	0.00	1.33	0.00	0.00	0.00	1.67	1.67	0.33	0.33	0.00	0.00	0.00	0.00	0.33	0.33	0.00	0.00	×	×	×	×
♀	Control	0.33	0.33	0.33	0.33	2.00	0.00	0.00	0.00	1.33	0.67	0.67	0.67	0.00	0.00	0.00	0.00	0.67	0.67	×	×	0.33	0.00	0.00	0.00
	Low dose	1.33	0.00	0.00	0.00	0.50	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	×	×	0.00	0.00	×	×
	Medium dose	0.33	1.00	1.00	0.67	1.50	0.00	0.00	0.00	1.67	1.67	0.00	0.00	1.33	0.00	0.00	0.00	1.00	1.00	×	×	0.00	0.00	×	×
	High dose	0.33	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.33	0.33	1.50	0.00	0.00	0.00	0.33	0.00	×	×	0.00	0.00	×	×
Satellite groups																									
♂	Control	0.00	1.33	1.33	0.00	2.00	0.00	1.33	0.00	1.33	1.33	0.67	0.67	0.00	0.00	0.33	0.00	1.33	1.33	0.33	0.00	×	×	×	×
	Low dose	0.00	1.33	1.33	0.00	2.33	0.00	1.67	0.00	2.33	2.33	1.33	1.33	0.00	0.00	1.00	1.00	1.33	1.33	0.00	0.00	×	×	×	×
	Medium dose	0.00	1.00	1.00	1.00	0.67	0.00	0.33	0.00	1.00	0.00	1.33	1.33	0.00	0.00	0.00	0.00	1.00	1.00	0.50	0.00	×	×	×	×
	High dose	0.00	0.00	0.00	1.33	0.00	0.00	1.00	0.00	1.00	0.67	0.33	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	×	×	×	×
♀	Control	0.00	0.67	0.67	0.00	1.33	0.00	1.67	0.00	1.00	1.00	0.67	0.67	0.00	0.00	0.00	0.33	0.33	0.33	×	×	1.00	0.00	×	×
	Low dose	0.00	1.33	1.33	0.00	1.67	0.00	1.00	0.00	2.33	2.33	1.67	1.67	0.00	0.00	0.67	0.00	2.00	2.00	×	×	0.67	0.00	×	×
	Medium dose	0.00	0.00	0.00	0.00	1.33	0.00	0.67	0.00	2.00	2.00	0.00	0.00	0.00	0.00	0.33	0.00	1.33	1.33	×	×	0.00	0.00	×	×
	High dose	0.00	0.00	0.00	1.00	1.33	0.00	0.00	0.00	1.67	1.33	0.00	0.00	0.00	0.00	1.50	0.00	1.67	1.67	×	×	0.00	0.00	×	×

Deg: degeneration; Nec: necrosis; ND: necrosis/degeneration; Inf: inflammation; Reg: regeneration; Pne: pneumonia; Bro: bronchitis; Pbro: peribronchitis; Oed: edema. Score: 0 = no damage, 1: >25% damage; 2: ≥25%–50% damage, 3: ≥50%–75% damage, and 4: ≥75% damage. ×: not evaluated.

induce temporary damage to the hematopoietic system. The hematological parameter can be used as an indicator to determine the toxic effect of compounds and provide pathophysiological information about mammals (Diaz *et al*., 2016). Erythrocyte levels showed a significant increase at the high dose compared to other groups in male treatment groups (Table 4). In the male satellite groups, erythrocyte levels in low and medium doses were not significantly different compared to those in the control group. A previous study also found an inconsistent pattern of increasing doses with hematological change (Chung *et al*., 2017). This result demonstrated that the recovery after *S. ligustrina*+DHP administration in hematological parameters of male mice was better than in female mice. Previous research found that male rats showed a fast recovery from acute nickel toxicity (Alcon *et al*., 1991). El Kabbaoui *et al*. (2017) found that female mice were more sensitive to hematological parameters than male mice after *C. ladaniferus* extract administration so the recovery may be slow.

Blood biochemical parameters were measured to evaluate the toxic effect on liver and kidney function because both organs are vital organs for survival (Nugroho *et al*., 2020). The treatment groups showed a significant increase in ALT and AST levels in male and female mice (Tables 6 and 7). Then, the level of ALT and AST decreased after the treatments were stopped for 2 weeks. It could be concluded that the liver damage after *S. ligustrina*+DHP treatments was self-repaired. ALT and AST are enzymes used as an indicator to evaluate liver damage (Tran and Tran, 2021). Both enzymes will be massively released into the bloodstream when a large number of liver cells are damaged (Al-Habori *et al*., 2002). The kidney function was evaluated via urea and creatinine measurement. The kidney is responsible for excreting metabolic waste and regulating osmotic and electrolyte concentrations (Tran and Tran, 2021). Creatinine is produced by the daily movement of muscle tissue, while urea is the end product of metabolism. A significant increase in creatinine, urea, Na^{2+}, and glucose levels in blood indicated the nephrotoxic effect of *S. ligustrina*+DHP administration, as supported by an increase in necrosis-degeneration and inflammation. The liver and kidney damage has been related to phytotherapeutics present in plants, such as alkaloids, phenol, hydroquinone, tannins, flavonoids, saponins, steroids, and terpenes, if administrated at a high dose (Evans, 1996; Manurung *et al*., 2019).

A histopathological examination was performed to evaluate microscopic damage to these organs. All groups showed more abnormalities (necrosis, degeneration, and inflammation) in the liver, kidneys, and ulcers than in other organs (Table 8). These results were confirmed by elevated ALT, AST, creatinine, and glucose levels in Tables 6 and 7. Although some parameters recovered after stopping treatment, organ damage was not completely repaired at the microstructure level. These findings may indicate that *S. ligustrina*+DHP had microstructure damage in the vital organs of mice.

CONCLUSION

In conclusion, subchronic oral administration of *S. ligustrina* and DHP for 28 days was safe in low doses (200 + 111 mg/kg BW). High doses (800 + 333 mg/kg BW) and medium doses (400 + 222 mg/kg BW) showed toxic effects including body weight, relative organ weight, hematology, biochemistry, and histopathology. However, the toxic effect of *S. ligestrina*+DHP was recovered after stopping treatments for 2 weeks.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

All procedures in the experiment were approved by the Animal Ethics Committee, IPB University (approval number: 154-2019).

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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