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Original article

Evaluation of sub-acute and sub-chronic toxicity of aqueous extract of *Coptosapelta flavescens* Korth. in a Swiss albino mice model



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ABSTRACT

Coptosapelta flavescens Korth. is one of the medicinal plants used as a traditional medicine in Vietnam for a long time. However, few documents proved its safety for a long time. Therefore, the study aimed to determine the toxicity effects of the aqueous extract of *C. flavescens* by determining its effects after sub-chronic oral administration in Swiss albino mice. The toxicity of *C. flavescens* extract was tested at doses of 400 and 1200 mg/kg, respectively, over 30 and 60 days on an *in vivo* model. Animal weight, behavior, and condition changes were monitored throughout the process. At the end of the observation periods, hematological indices and histopathological features of the animal specimens were analyzed. The administration of *C. flavescens* extract at doses of 400 and 1200 mg/kg caused a weight difference in male mice after 30 days and in both sexes after 60 days of administration compared to the control group. There were no significant changes in hematological parameters and biochemical indexes of the liver and kidney compared with the control group. The histological structure of the liver showed changes when treated with *C. flavescens* at both doses after 60 days, whereas the histological structure of the kidneys exhibited differences only at the dose of 400 mg/kg. Overall, the findings of this study indicate that *Coptosapelta flavescens* Korth. is unlikely to have subacute and subchronic oral toxicity. However, there are some minor effects on the liver and kidneys.

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1. Introduction

Plant-based products have been used for medicinal purposes for a long time. Until now, it is estimated that >80% of the world's population is dependent on medicinal herbs or products of plant origin for their health protection needs (James T. Mukinda & Eagles, 2010). In developing countries, most people believe in traditional medicines because of their availability, accessibility, and effectiveness, which is a part of their culture. Herbal remedies are also considered an alternative to orthodox pharmaceutical therapies in

these countries. These traditional remedies are recognized to potentially treat diseases such as arthritis, osteoporosis, kidney and liver, cardiovascular disorders, and diabetes diseases (Kunle O.F. et al., 2012). In addition, it has been suggested that herbal remedies are safer and less harmful to the human body than synthetic drugs because they are of natural origin (Unuofin et al., 2018). For these reasons, the World Health Organization (WHO) has recognized that traditional medicine is essential in treating diseases and encourages member states to develop appropriate policies for control and development (WHO, 2005). However, although drugs from plants are widely used, their effectiveness is rarely scientifically evaluated. In addition, patients often cannot distinguish the similarities and differences between herbal and approved drugs. Many people think herbs are natural alternatives to synthetic drugs but do not realize they are biologically active compounds. In particular, some compounds can be toxic to humans if used in excess. Many studies have reported the toxic effects on the human body of medicinal herbs (El Hilaly et al.,

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2004; Taziebou et al., 2007). Therefore, evaluating and developing standards for each remedy to detect and control undesirable effects when treating human diseases is necessary (see Fig. 1).

Coptosapelta flavescens Korth. is a medicinal herb found and used for a long time by many ethnic groups in Southeast Asian countries as traditional medicine. There have been several reports documenting the effectiveness of using this medicinal herb in the treatment of diseases and health care, such as used as a skin cream, in the treatment of helminths, abdominal pain, and nasal ulcers, antipyretic and anti-rheumatic (Bremer & Eriksson, 2009; Lin, 2005). In addition, the extract from *C. flavescens* was reported to have the ability to inhibit bacteria more effectively than metronidazole, treat dysentery, lower blood pressure, anti-inflammatory, treatment of malaria (Houkong et al., 2015; Kongyen et al., 2014; Kosala et al., 2017, 2018; Sutomo, 2017). Some studies analyzing the chemical composition of *C. flavescens* have recorded the presence of anthraquinones, saponins, and diverse phytochemical compounds (Nguyen et al., 2020). Thereby basically verifying that the effectiveness of this medicinal herb is closely related to the biological compounds available in the plant.

According to the traditional folk experience of the Hre ethnic group in central Vietnam, *C. flavescens* root extract is used to disinfect wounds, promote skin recovery, treat colds and flu, and support disease treatment, Gout. Furthermore, this medicinal herb is processed into a product known as “Cao Khai,” which is used as a traditional medicine to treat bone and joint diseases, anti-inflammatory, antibacterial, and diarrhea cure.

A primary concern regarding the therapeutic use of natural products is the lack of scientific evidence to support reported uses based solely on traditional knowledge. To our knowledge, several studies have been conducted on the acute toxicity of *C. flavescens* extract (Pham et al., 2022). However, these studies only performed the contents individually. In particular, the toxicity reports of this plant have only published results that are limited to the acute level, without detailing immediate changes in the test animals such as weight change, behavioral manifestations, hematological profile, and histological analysis. Moreover, the studies only stopped at the acute level but were not conducted at the subacute, chronic, or later levels. This is a flaw in previous studies, making it difficult to make an overall assessment of the safety of this medicinal herb.

Therefore, a detailed scientific data set on the subacute and subchronic toxicity of the aqueous extract of *C. flavescens* collected in Vietnam will be provided in this study. Accordingly, this study evaluates toxicity in an *in vivo* model of *Swiss albino* mice in a two-stage subacute and subchronic (30 days and 60 days) formulation. The study provides data on the changes in test subjects after

using *C. flavescens* extracts, including body weight, changes in hematological parameters, biochemical parameters, and microscopic morphology. These results will be the basis for evaluating the effects of *C. flavescens* when used for a long time on the human body in the future.

2. Materials and methods

2.1. Plant material collection and extraction

Roots of *C. flavescens* were collected in September 2020 in Ninh Son district, Ninh Thuan Province, Vietnam. Specimens were identified and authenticated by the Oriental Medicine Association of Ninh Thuan Province, Vietnam. A sample form has been kept at the Faculty of Pharmacy, Nguyen Tat Thanh University, Vietnam. Raw materials are dried in the shade for one week, and then preliminary treatment by removing leaves and cutting them into small pieces ($d = 0.5$ mm). The ingredients are then ground into a fine powder in a blender and stored in sealed polyester bags. *C. flavescens* root powder was mixed with water (1:3 ratio) and then boiled at $100\text{ }^{\circ}\text{C}$ for 48 h. The extraction process was repeated three times to obtain the liquid solution. The solution was then concentrated until the moisture content reached below 10%. Finally, the resulting dried extract was vacuum-sealed and stored in sealed polyester bags at room temperature.

2.2. Animals

Mice, male and female (50% each), *Swiss albino* strain, six weeks old, weighing 18–24 g were used in the study, provided by the Institute of Vaccines and Medical Biologicals Nha Trang, Khanh Hoa Province, Vietnam. Animals were kept in polypropylene cages (separately raised for males and females) with $25 \times 35 \times 15$ cm dimensions containing sterilized rice husks as a cage lining material. The animals were healthy and showed no abnormalities, and they were provided adequate drinking water and food with rice flour, soybeans, corn, and vitamins. The study was conducted according to the “Guidelines for pre-clinical and Clinical Trials of Traditional Medicines and Herbal Medicines” issued by the Ministry of Health (Vietnam) under Decision No. 141/QĐ-K2ĐT dated October 27th/ 2015. The study was conducted as part of a scientific research project under the authority of the Department of Science and Technology of Ninh Thuan province, Vietnam (Code: 12/2020/HĐ-SKHCN) and was approved for ethical research to implement animal assessment.

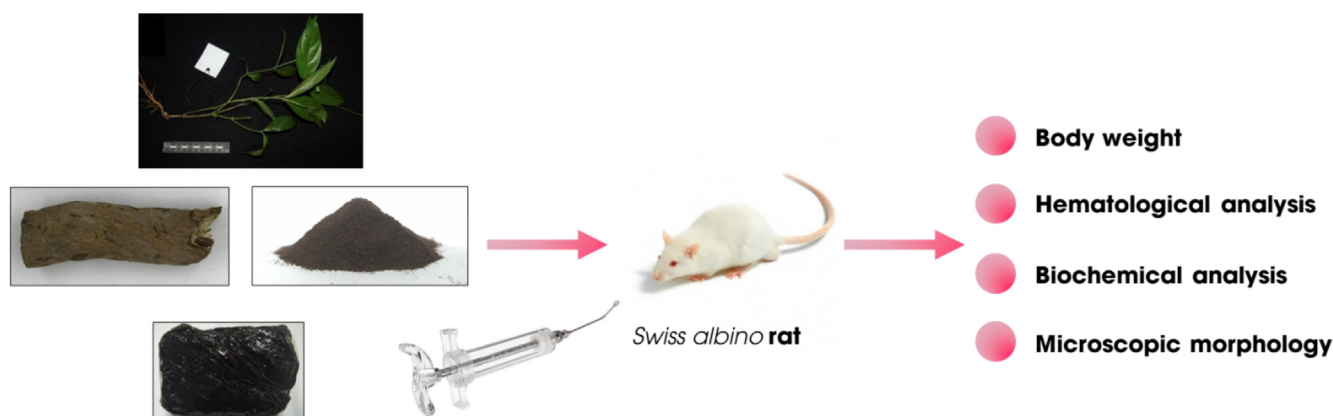


Fig. 1. Schematic representation of subacute and subchronic toxicity of *C. flavescens* extract in *Swiss albino* mice model.

2.3. Experimental design for subacute toxicity study

- Group I: Control
- Group II: Control after 30 days: distilled water, dose 10 ml/kg/day for 30 consecutive days
- Group III: *C. flavescens* extract, the dose of 400 mg/kg for 30 consecutive days
- Group IV: *C. flavescens* extract, the dose of 1200 mg/kg for 30 consecutive days
- Group V: Distilled water, dose 10 ml/kg/day for 60 consecutive days
- Group VI: *C. flavescens* extract, the dose of 400 mg/kg for 60 consecutive days
- Group VII: *C. flavescens* extract, the dose of 1200 mg/kg for 60 consecutive days

Mice were continuously treated with distilled water or sample daily (volume 10 ml/kg/time) in the morning (8---10 am) for 30 or 60 days, depending on the experimental layout. The animals fasted for two hours before drinking. Mice in group I played the role of control and were slaughtered after 05 days to investigate the parameters of the mice before the test. Similarly, mice in groups II and V were used to control for fluctuations in control indices after 30 and 60 days, respectively. The remaining mice were divided into two groups to receive *C. flavescens* extract (dose 400 and 1200 mg/kg) for 30 days (groups III and IV) and 60 days (groups VI and VII). Changes in the body weight of mice were recorded by weighing the mice once a week. The general behavior and responses of the mice were observed continuously.

2.4. Hematological and biochemical analysis

After 30 or 60 days, mice were anesthetized with CO₂ stone and quickly operated to take blood from the heart to perform tests on hematological indicators such as red blood cell count (RBC), white blood cell count (WBC), and white blood cell formula, platelet count (PLT), hemoglobin (HGB), erythrocyte hematocrit (HCT), mean red blood cell volume (MCV) (hematopoietic function test) using ABX MICROS hematology 60 (Horiba Medical, France). Check liver function (aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, and total cholesterol); renal function (urea, creatinine) was checked by Cobas C501 automatic biochemical analyzer and corresponding test kit (Roche, Germany). Analytical parameters were performed on the same blood sample. The collected blood samples were transferred into specialized tubes and kept at a temperature between 2 and 8 °C until analysis, which was performed within a maximum of 2 h after collection. (Serfilippi et al., 2003; Danneman et al., 2000).

2.5. Histopathological examination

On day 60, observe and record the changes in the macroscopic and internal organs. Then, the liver and kidneys were washed with cold control saline and dried. Next, the liver and kidney were immersed in 10% formalin. Six liver and kidney samples in one group were randomly collected for microscopic analysis after hematoxylin-eosin (HE) staining by optical microscopy (Labomed, USA). The degree of inflammation and liver damage was assessed according to the HAI-Knodell scale (HAI - hepatitis activity index) with an 18-point scale based on the degree of necrosis around the portal space (0–4 points); necrosis around the central vein (0–6 points); necrosis in the lobules (0–4 points); periportal inflammation (0–4 points) (Brunt, E.M., 2000). If, during the test, a mouse dies, consider the condition of the internal organs through anatomy.

2.6. Statistical analysis

The results are presented as mean ± SEM (standard error of the mean). The differences between the lots were analyzed by Kruskal-Wallis and Mann-Whitney tests with SPSS 22.0 software. The difference was statistically significant when p < 0.05.

3. Results

3.1. Sub-acute and sub-chronic toxicity study

A subacute toxicity study of *C. flavescens* extract was conducted according to “Guidelines for pre-clinical and clinical trials of oriental medicines and herbal medicines” issued by the Ministry of Health (Vietnam) under Decision No. 141/QD-K2DT dated October 27, 2015. All study animals were treated daily with *C. flavescens* extract at study doses (400 and 1200 mg/kg), and they all survived the entire experiment. The group of mice treated with the extract at 30 and 60 days did not observe any toxicity when compared to the initial control group of mice.

3.2. General condition and weight change

All mice in the lots had smooth hair and bright eyes; they performed normal activities, eating and cleaning. Two groups of mice treated with high oral doses of *C. flavescens* extract, 400 mg/kg and 1200 mg/kg, had 2/10 mice with lymphadenopathy in the neck region. The body weights of mice during the test are presented in Table 1.

The results show that control mice gain weight steadily from 2 to 5 g after each week. The group treated with *C. flavescens* extract at doses of 400 mg/kg and 1200 mg/kg had an increase in body weight of about 2–3 g per week, a lower increase than the control

Table 1
Effect of *C. flavescens* extract on body weight.

Period	Average weight ± SEM (g)			
	Group Sex	Control	400 mg/kg	1200 mg/kg
Day 0	Both	23.5 ± 0.2	23.0 ± 0.4	23.0 ± 0.4
	Male	23.9 ± 0.3	23.7 ± 0.4	22.5 ± 0.5
	Female	23.1 ± 0.4	22.2 ± 0.6	23.5 ± 0.5
Day 7	Both	28.2 ± 0.5	25.6 ± 0.6*	25.8 ± 0.4**
	Male	28.0 ± 0.7	27.4 ± 0.6##	26.2 ± 0.4*
	Female	28.4 ± 0.8	23.9 ± 0.6**	25.4 ± 0.8*
Day 14	Both	31.3 ± 0.6	28.7 ± 0.4**	28.5 ± 0.5**
	Male	32.5 ± 0.7	29.2 ± 0.5**	28.6 ± 0.6**
	Female	30.2 ± 0.7	28.2 ± 0.7	28.5 ± 0.7
Day 21	Both	31.7 ± 0.6	30.6 ± 0.5	31.0 ± 0.6
	Male	36 ± 0.6	31.7 ± 0.7	31.4 ± 0.6
	Female	30.8 ± 0.9	29.5 ± 0.7	30.6 ± 1.1
Day 28	Both	34.2 ± 0.6	32.5 ± 0.6	32.9 ± 0.6
	Male	35.8 ± 0.5##	34.0 ± 0.6##	34.5 ± 0.8##
	Female	32.6 ± 0.8	31.1 ± 0.7	31.3 ± 0.7
Day 35	Both	39.3 ± 1.1	36.8 ± 0.7	37.8 ± 1.0
	Male	40.3 ± 0.9	38.0 ± 0.6	39.9 ± 0.6*
	Female	38.2 ± 2.1	35.6 ± 1.0	35.7 ± 1.4
Day 42	Both	41.8 ± 1.4	38.1 ± 0.8*	39.2 ± 1.1
	Male	44.2 ± 0.7	40.2 ± 0.5**##	41.3 ± 0.8**
	Female	39.4 ± 2.2	36.1 ± 0.7	37.1 ± 1.5
Day 49	Both	44.1 ± 1.5	39.8 ± 0.8*	41.4 ± 1.2
	Male	46.6 ± 0.8	41.5 ± 0.9**	44.2 ± 0.7
	Female	41.6 ± 2.4	38.0 ± 0.9	38.7 ± 1.7
Day 56	Both	46.4 ± 1.4	40.4 ± 0.7**	42.1 ± 1.3*
	Male	48.6 ± 1.1	41.0 ± 0.8**	45.0 ± 0.7*
	Female	44.2 ± 2.3	39.8 ± 1.1	39.1 ± 1.7*

* : p < 0.05 and ** : p < 0.01 when compared with control group at the same time.
: p < 0.05 and ## : p < 0.01 when comparing between two sexes in the same group.

group. The mean weight of the group treated with the 400 mg/kg dose was statistically significantly lower than that of the control mice at the same time on days 7, 14, 42, 49, and 56 ($p < 0.05$). In the group treated with 1200 mg/kg, the body weight of mice was significantly lower than that of the control group on days 7, 14, and 56 ($p < 0.05$). When compared in each group, control male and female mice had no significant difference in weight ($p > 0.05$). In contrast, the body weight of female mice treated with the extract was lower than that of male mice of the same batch, with statistically significant differences at day 28 and day 42 ($p < 0.05$).

3.3. Hematological analysis

The hematological parameters are recorded and presented in Table 2. First, the parameters related to leukocytes: after 30 days, the mice group treated with *C. flavescens* extract at a 400 mg/kg dose had no significant changes in leukocyte parameters compared to the control group ($p > 0.05$). In contrast, the group treated with the 1200 mg/kg dose had a statistically significant lower total white blood cell count and lymphocyte count ($p < 0.05$). After 60 days, the white blood cell counts in two groups of mice treated with *C. flavescens* extract were higher than those in the control

Table 2
The changes in hematological parameters of mice after 30 days and 60 days of treatment with *C. flavescens* extract.

Parameter	Group	After 30 days			After 60 days		
		Control	400 mg/kg	1200 mg/kg	Control	400 mg/kg	1200 mg/kg
WBC ($10^9/L$)	Both	12.9 ± 0.8	11.7 ± 1.0	9.9 ± 0.8*	8.8 ± 1.1	11.0 ± 1.2	12.7 ± 1.3*
	Male	13.5 ± 1.0	13.0 ± 1.5	11.9 ± 1.1#	6.0 ± 0.4#	9.3 ± 1.7*	9.5 ± 1.0**##
	Female	12.3 ± 1.2	10.4 ± 1.1	7.9 ± 0.4*	11.6 ± 1.3	12.6 ± 1.5	16.0 ± 1.1**
Neutrophil count ($10^9/L$)	Both	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.7 ± 0.1**	0.7 ± 0.1**
	Male	0.4 ± 0.0#	0.6 ± 0.2	0.6 ± 0.2	0.2 ± 0.0	0.7 ± 0.1**	0.8 ± 0.1**
	Female	0.7 ± 0.1	0.4 ± 0.0	0.3 ± 0.1*	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Lymphocyte count ($10^9/L$)	Both	11.0 ± 0.7	9.7 ± 0.8	8.1 ± 0.7*	7.5 ± 0.9	8.7 ± 1.1	10.6 ± 1.2*
	Male	12.0 ± 0.9	10.6 ± 1.2	9.6 ± 0.9#	5.2 ± 0.4#	6.8 ± 1.2	7.4 ± 0.8**##
	Female	9.9 ± 1.0	8.8 ± 1.1	6.6 ± 0.4*	9.8 ± 1.2	10.5 ± 1.4	13.7 ± 1.0*
Monocyte count ($10^9/L$)	Both	1.4 ± 0.2	1.5 ± 0.2	1.3 ± 0.3	1.0 ± 0.2	1.7 ± 0.2	1.4 ± 0.1
	Male	1.1 ± 0.1	1.7 ± 0.5	1.6 ± 0.5	0.6 ± 0.0#	1.8 ± 0.4**	1.3 ± 0.1**#
	Female	1.6 ± 0.4	1.2 ± 0.1	1.0 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	1.6 ± 0.1
RBC ($10^{12}/L$)	Both	10.05 ± 0.15	9.23 ± 0.25*	8.94 ± 0.29**	10.04 ± 0.11	10.45 ± 0.21	9.98 ± 0.13
	Male	9.94 ± 0.18	9.13 ± 0.37	8.77 ± 0.53*	10.00 ± 0.21	10.27 ± 0.28	10.02 ± 0.18
	Female	10.15 ± 0.24	9.33 ± 0.37	9.10 ± 0.26*	10.08 ± 0.10	10.63 ± 0.31	9.94 ± 0.22
Hgb (g/dL)	Both	14.0 ± 0.3	13.4 ± 0.4	12.8 ± 0.5*	13.9 ± 0.1	14.3 ± 0.3	14.0 ± 0.2
	Male	13.8 ± 0.4	13.3 ± 0.5	12.5 ± 1.0	13.9 ± 0.1	14.0 ± 0.4	13.9 ± 0.2
	Female	14.1 ± 0.5	13.4 ± 0.6	13.1 ± 0.3	14.0 ± 0.2	14.5 ± 0.4	14.0 ± 0.4
HCT (%)	Both	52.2 ± 1.1	49.9 ± 1.3	47.1 ± 2.0*	53.7 ± 0.7	52.7 ± 1.2	50.6 ± 1.0*
	Male	51.8 ± 1.0	49.7 ± 1.3	47.5 ± 3.8	54.8 ± 0.8	52.3 ± 1.5	51.4 ± 0.8*
	Female	52.6 ± 2.0	50.2 ± 2.4	46.7 ± 1.8*	52.6 ± 0.8	53.0 ± 1.7	49.9 ± 1.8
MCV (fL)	Both	52 ± 1	54 ± 1	53 ± 1	53 ± 1	51 ± 1**	51 ± 1*
	Male	52 ± 1	55 ± 1	54 ± 2	55 ± 1#	51 ± 0**	51 ± 1*
	Female	52 ± 2	54 ± 1	51 ± 2	52 ± 1	50 ± 1	50 ± 1
PLT ($10^9/L$)	Both	1198 ± 78	1035 ± 59	1203 ± 55	990 ± 43	1133 ± 79	1060 ± 57
	Male	1052 ± 111	1071 ± 88	1202 ± 106	995 ± 61	1107 ± 103	979 ± 42
	Female	1344 ± 65	999 ± 84*	1205 ± 48	986 ± 72	1160 ± 125	1140 ± 97

WBC: White Blood Cell; RBC: Red Blood Cell; Hgb: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscle Volume; PLT: Platelet Count.

* : $p < 0.05$ and ** : $p < 0.01$ when compared with control group at the same time.

: $p < 0.05$ and ## : $p < 0.01$ when comparing between 2 sexes in the same group.

Table 3
The changes in liver parameters of mice after 30 days and 60 days of treatment with *C. flavescens* extract.

Parameter	Group	After 30 days			After 60 days		
		Control	400 mg/kg	1200 mg/kg	Control	400 mg/kg	1200 mg/kg
AST (U/L)	Both	76.37 ± 6.20	77.68 ± 5.30	76.56 ± 6.50	90.29 ± 5.72	106.77 ± 6.80	91.11 ± 3.02
	Male	64.31 ± 2.70 [#]	69.58 ± 6.31	69.71 ± 12.24	80.62 ± 7.48	96.15 ± 9.23	93.42 ± 6.04
		Female	88.42 ± 9.65	85.79 ± 7.33	83.40 ± 4.14	99.97 ± 6.88	117.39 ± 10.31
	Both		38.11 ± 3.48	37.24 ± 2.66	33.50 ± 2.13	45.58 ± 4.64	46.14 ± 7.33
	Male	38.83 ± 6.19	33.97 ± 3.88	34.25 ± 2.98	51.54 ± 7.50	42.46 ± 2.05	46.23 ± 3.92 [#]
		Female	37.39 ± 3.98	40.51 ± 3.38	32.76 ± 3.35	39.62 ± 4.65	49.82 ± 13.93
Bilirubin (µmol/L)	Both		1.62 ± 0.21	1.62 ± 0.19	1.71 ± 0.16	1.90 ± 0.26	1.52 ± 0.29
	Male	1.88 ± 0.34	1.64 ± 0.30	2.04 ± 0.14 [#]	2.53 ± 0.21 [#]	1.97 ± 0.51	1.87 ± 0.33
		Female	1.36 ± 0.21	1.59 ± 0.28	1.37 ± 0.21	1.27 ± 0.51	1.06 ± 0.36
	Both		41.90 ± 1.39	38.82 ± 1.48	38.00 ± 2.11	49.27 ± 1.40	44.97 ± 0.80 [*]
	Male	45.19 ± 0.74 [#]	38.22 ± 2.52 ^{**}	37.16 ± 3.33 ^{**}	52.21 ± 1.16 [#]	45.18 ± 1.52 [*]	46.48 ± 1.49 [*]
		Female	38.60 ± 1.65	39.43 ± 1.82	38.84 ± 2.94	46.34 ± 1.74	44.76 ± 0.85
Cholesterol (mmol/L)	Both		2.34 ± 0.16	2.34 ± 0.17	2.59 ± 0.20	2.93 ± 0.10	2.56 ± 0.13 [*]
	Male	2.61 ± 0.21	2.59 ± 0.26	2.61 ± 0.33	3.12 ± 0.10	2.66 ± 0.23	3.08 ± 0.07
		Female	2.07 ± 0.19	2.09 ± 0.18	2.57 ± 0.25	2.74 ± 0.15	2.46 ± 0.18

ALT: Alamin amino transferase; AST: Aspartat amino transferase;
* : p < 0.05 and ** : p < 0.01 when compared with control group at the same time.

Table 4
The changes in renal parameters of mice after 30 days and 60 days of treatment with *C. flavescens* extract.

Parameter	Group	After 30 days			After 60 days			
		Control	400 mg/kg	1200 mg/kg	Control	400 mg/kg	1200 mg/kg	
Urea (mmol/L)	Both	5.43 ± 0.59	4.34 ± 0.43	5.36 ± 0.51	8.12 ± 0.51	5.17 ± 0.52 ^{**}	9.72 ± 0.39 [*]	
	Male	4.93 ± 0.85	4.25 ± 0.62	5.01 ± 0.69	7.10 ± 0.53 [#]	5.66 ± 0.59	9.45 ± 0.57 [*]	
		Female	5.93 ± 0.85	4.43 ± 0.65	5.70 ± 0.81	9.15 ± 0.76	4.69 ± 1.39 ^{**}	9.99 ± 0.56
	Creatinin (µmol/L)		Both	28.74 ± 1.12	27.76 ± 1.62	30.04 ± 1.38	34.82 ± 1.33	30.97 ± 1.42 [*]
		Male	29.04 ± 1.16	28.07 ± 2.62	29.15 ± 2.46	34.44 ± 1.47	30.18 ± 1.29 [*]	35.70 ± 3.80
			Female	28.43 ± 2.06	27.45 ± 2.22	30.94 ± 1.46	35.20 ± 2.68	31.75 ± 2.41

* : p < 0.05 and ** : p < 0.01 when compared with control group at the same time.

Table 5
Results of microscopic analysis of liver cell structure of groups of mice.

Group (n = 6)	Control	400 mg/kg	1200 mg/kg
Liver microscopy			
Normal	2/6	0/6	0/6
Minimal hepatitis	4/6	4/6	6/6
Moderate hepatitis	0/6	2/6	0/6

group at the same time. The number of neutrophils in the group treated with the 400 mg/kg dose and the total white blood cells, neutrophils, and lymphocytes in the group treated with the 1200 mg/kg dose increased statistically significantly (p < 0.05).

However, the leukocyte parameters of the tested mice were within the normal range of the mice. When comparing the two sexes in the same group, the results showed that the control group and the group treated with the 400 mg/kg extract had the most leukocyte parameters, not significantly different between male and female mice in the same group (p > 0.05). In the group treated with a dose of 1200 mg/kg, after 30 days, female mice had lower total white blood cells and lymphocytes than male mice (p < 0.05). However, after 60 days, the female mouse group had more total white blood cells, lymphocytes, and monocytes than male mice (p < 0.05).

About erythrocyte-related parameters after 30 days of testing, the RBC index in the group treated with the extract dose of

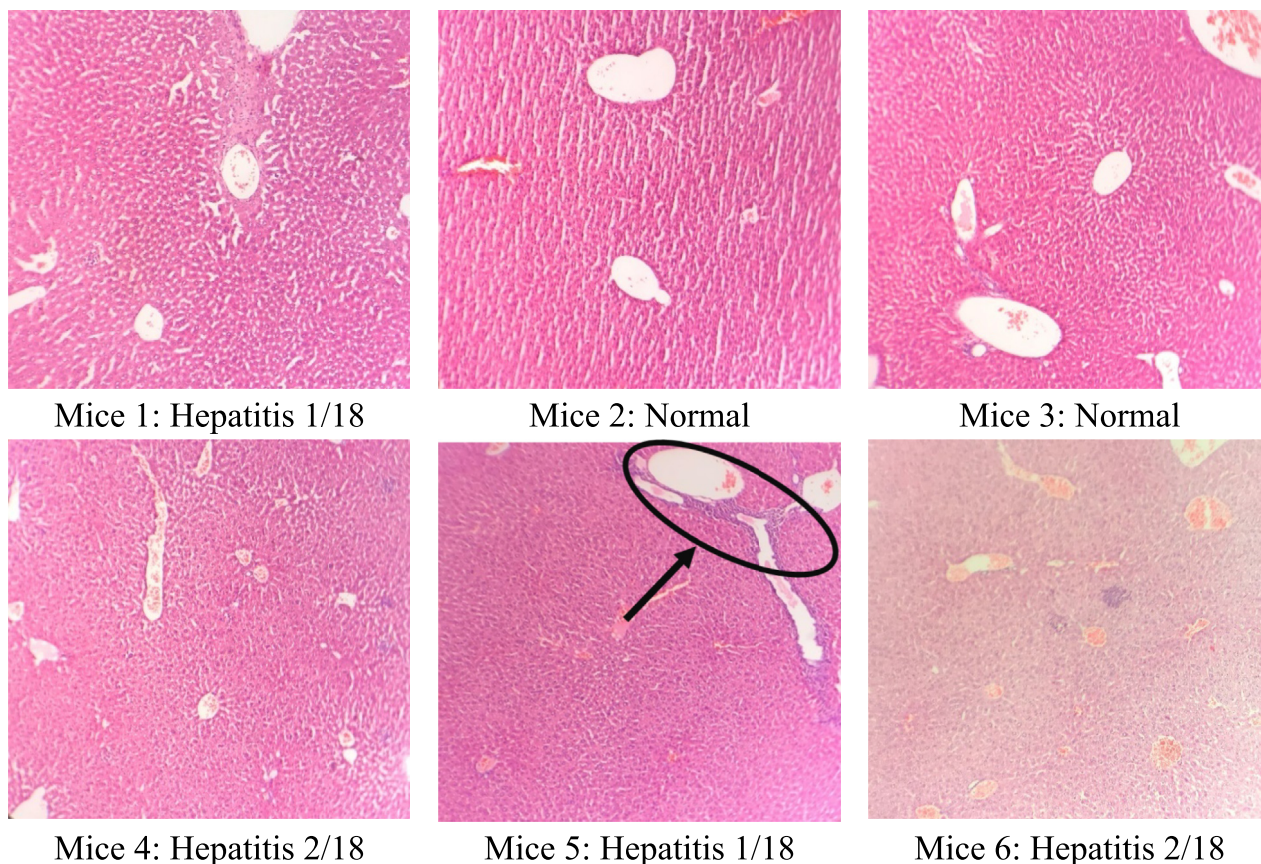


Fig. 2. Microstructure of mice hepatocytes in the control group.

400 mg/kg and the RBC, HgB, and HCT indexes in the group treated with the 1200 mg/kg dose were statistically significantly lower than that of control mice at the same time ($p < 0.05$). After 60 days, the parameters of red blood cells in mice treated with 400 mg/kg dose did not change significantly compared with control mice. The 1200 mg/kg dose group decreased HCT and MCV compared to the control group ($p < 0.05$). However, these parameters are all within the normal range of mice (HCT: 40 – 55 %; MCV: 48 – 56 fL) (Serfilippi et al., 2003; Suckow M.A., Danneman P., 2001). Red blood cell flow parameters between the two sexes in the same experimental group did not significantly differ from one another ($p > 0.05$).

Regarding platelet count, the group of mice that received 400 mg/kg and 1200 mg/kg extracts for 30 days and 60 days had no significant difference in platelet count (PLT) compared with the control group at the same time survey ($p > 0.05$). The platelet counts of the two groups of male and female mice did not significantly differ when compared within the same experimental group in any group ($p > 0.05$).

3.4. Liver function analysis

The liver parameters are recorded and presented in Table 3. The results showed that after 30 and 60 days of testing, the group of mice treated with extracts at doses of 400 mg/kg and 1200 mg/kg had no significant changes in liver function parameters (AST, ALT, total bilirubin, albumin, and cholesterol levels) compared with the control group ($p > 0.05$). Notably, the index of albumin and total cholesterol in the group of mice taking the extract at a dose of 400 mg/kg for 60 days had a statistically significant decrease compared with control mice at the same time ($p < 0.05$). Most liver

function indexes were not significantly different between the sexes when compared within the same experimental group. Although there is a difference between male and female mice in some indicators, this difference is random, does not follow a particular trend, and occurs in the whole control group. Physiological differences between individual mice can explain this difference.

3.5. Kidney function analysis

The results of the parameters to evaluate kidney function are presented in Table 4. After 30 days of testing, the urea and creatinine indexes of the group of mice treated with *C. flavescens* extract at doses of 400 mg/kg and 1200 mg/kg had no significant difference compared with the control group ($p > 0.05$). After 60 days, the group of mice taking the extract at a dose of 400 mg/kg had statistically significant reductions in urea and creatinine parameters ($p < 0.05$). The group taking an extract dose of 1200 mg/kg had a high urea index ($p < 0.05$), while the creatinine index was similar compared with the control group at the same time. However, the urea and creatinine results of the mice in the experimental groups were within the normal range. The renal function indexes between the sexes in the same experimental group were not significantly different ($p > 0.05$).

3.6. Microscopic liver morphology analysis

The results of the microscopic analysis of mice liver cells are presented in Table 5 and Figs. 2, 3, and 4. The control group had 2/6 normal liver samples, 4/6 minimal hepatitis samples with an HAI score of 1/18, and 2/18 with intralobular necrosis. This may be because, after 60 days, the mice's hepatocyte structure changed

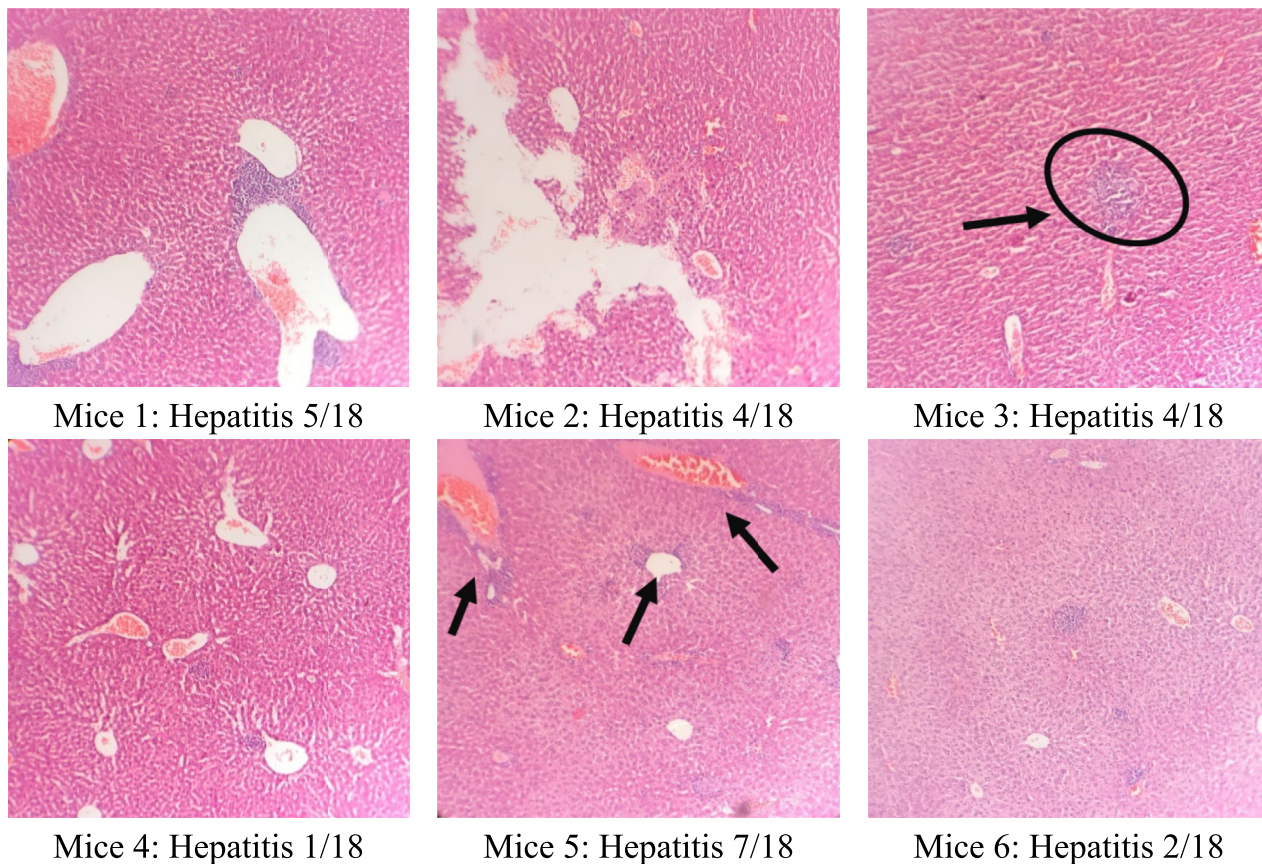


Fig. 3. The microstructure of mice liver cells in the group taking *C. flaveszens* extract dose of 400 mg/kg.

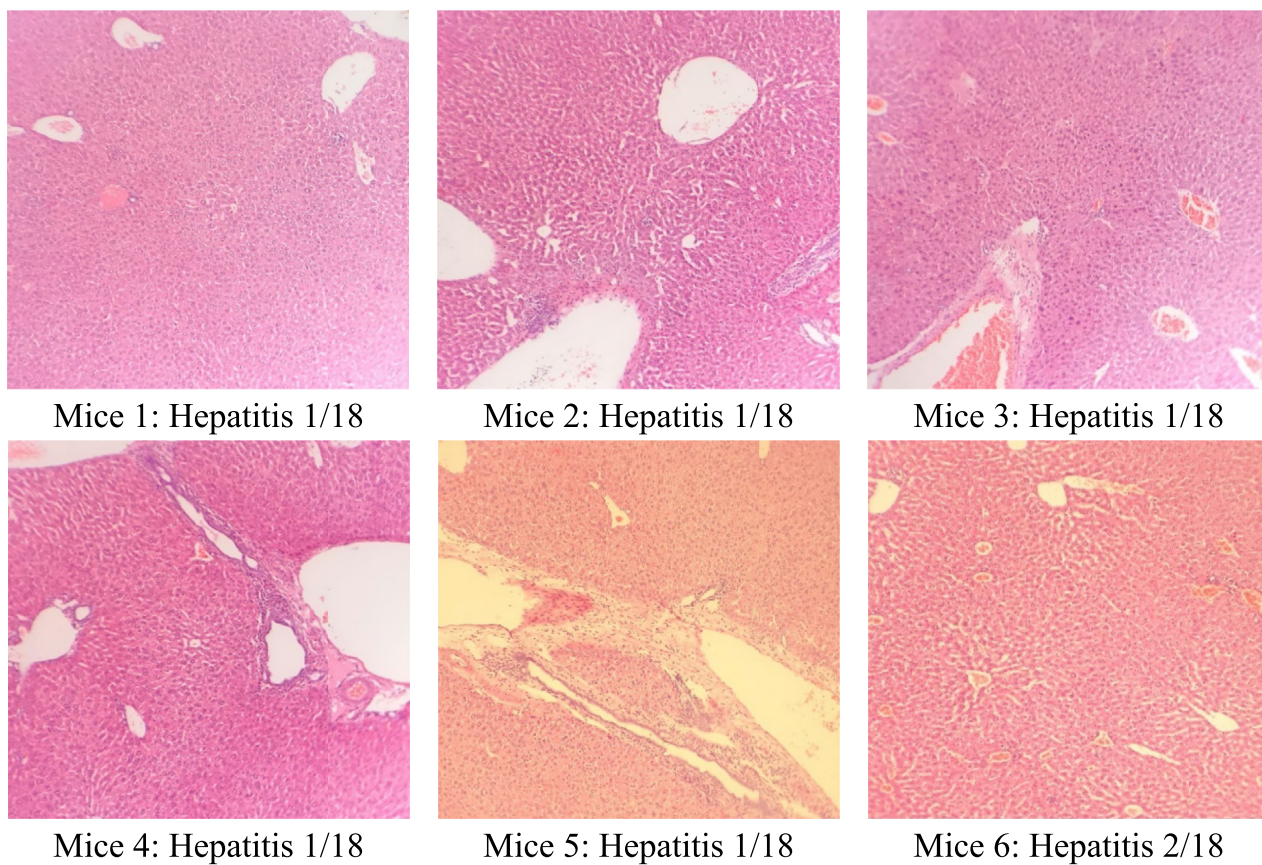


Fig. 4. The microscopic structure of mice liver cells in high oral *C. flaveszens* dose 1200 mg/kg.

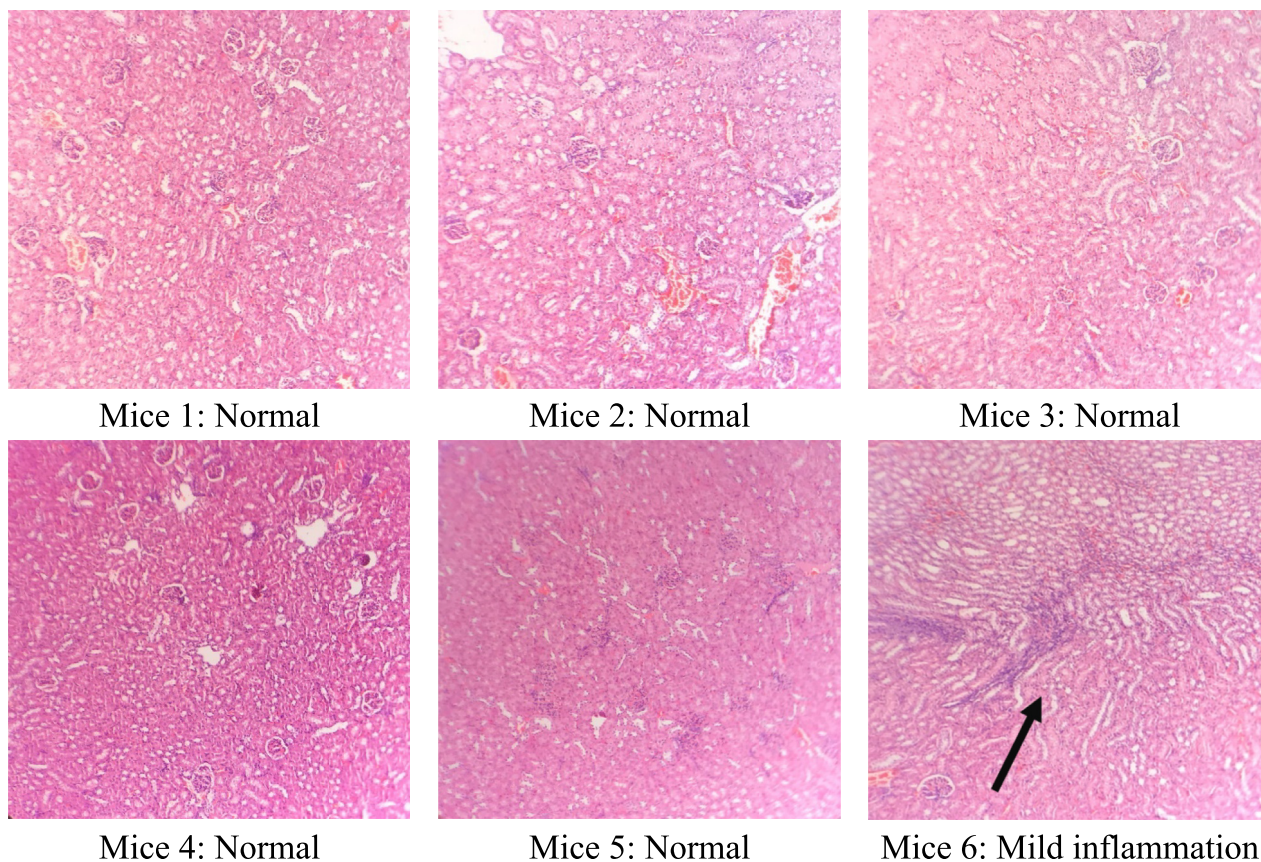


Fig. 5. Microstructure of mice kidney cells in the control group.

Table 6
Results of microscopic analysis of kidney cell structure of mouse groups.

Group (n = 6)	Control	400 mg/kg	1200 mg/kg
Renal microstructure			
Normal	5/6	0/6	6/6
Nephritis - mild pyelonephritis	1/6	6/6	0/6

over time. In the group that treated *C. flavescens* extract 400 mg/kg for 60 days, 4/6 samples had minimal hepatitis with an HAI score between 1/18---4/18, and 2/6 samples with moderate hepatitis with a score of HAI were 5/18 and 7/18, respectively. Samples showed periportal necrosis, periportal necrosis, intralobular necrosis, and periportal inflammation. The group that treated the extract at a 1200 mg/kg dose had 6/6 samples of minimal hepatitis with a score of 1/18---2/18.

3.7. Effect of plant extracts on microscopic kidney morphology

The results of the microscopic analysis of the kidney cells of the tested mice after 60 days are presented in Table 6 and Figs. 5–7. After 60 days of testing, the control group had 5/6 normal kidney samples and 1/6 mild pyelonephritis - pyelonephritis. The mice group treated with the extract at a dose of 400 mg/kg had 6/6 samples of mild nephritis - pyelonephritis with the phenomenon of infiltration of many lymphocytes and plasma cells in the interstitial peritubular and renal tubules. In addition, a small number of renal tubules were reported to be destroyed. All renal samples in the 1200 mg/kg dose group showed normal renal microstructure.

4. Discussion

Extract from *C. flavescens* is a traditional alternative medicine used to treat various illnesses. The vast majority of rural communities believe herbal preparations and remedies are secure and efficient, which encourages their indiscriminate use. At the same time, the lack of scientific data will trigger the long-term use of these remedies without controlling the dose and concentration. From there, it can cause side effects or harm the patient’s body. Therefore, an analysis of oral toxicity is needed to observe the effects on the body and will help determine a safe dose range for further studies.

The present study evaluated the subacute toxicity of *C. flavescens* extract in animal models. No deaths were reported after 400 or 1200 mg/kg oral administration for 30 days and 60 days. The animals did not change their behaviour during the test at 30 days and 60 days. In addition, there were no notable modifications in food or water intake in the tested mice during 30 and 60 days, which indicates that the animals can tolerate food and water.

Body weight changes indicate an animal’s control and pathological health status. Weight change is a toxicity marker in laboratory animals, as determined by toxicity assays (Unuofin et al., 2018). In this trial, the use of *C. flavescens* extracts at doses of 400 and 1200 mg/kg induced a slight effect on the body weight of mice, reducing the degree of weight gain compared with the control group. The difference in body weight between the two sexes of animals can be observed, which may be attributed to a combination of factors, including the absorption of the plant extract and inherent physiological differences among mice, resulting in slight changes in the animals’ eating habits.

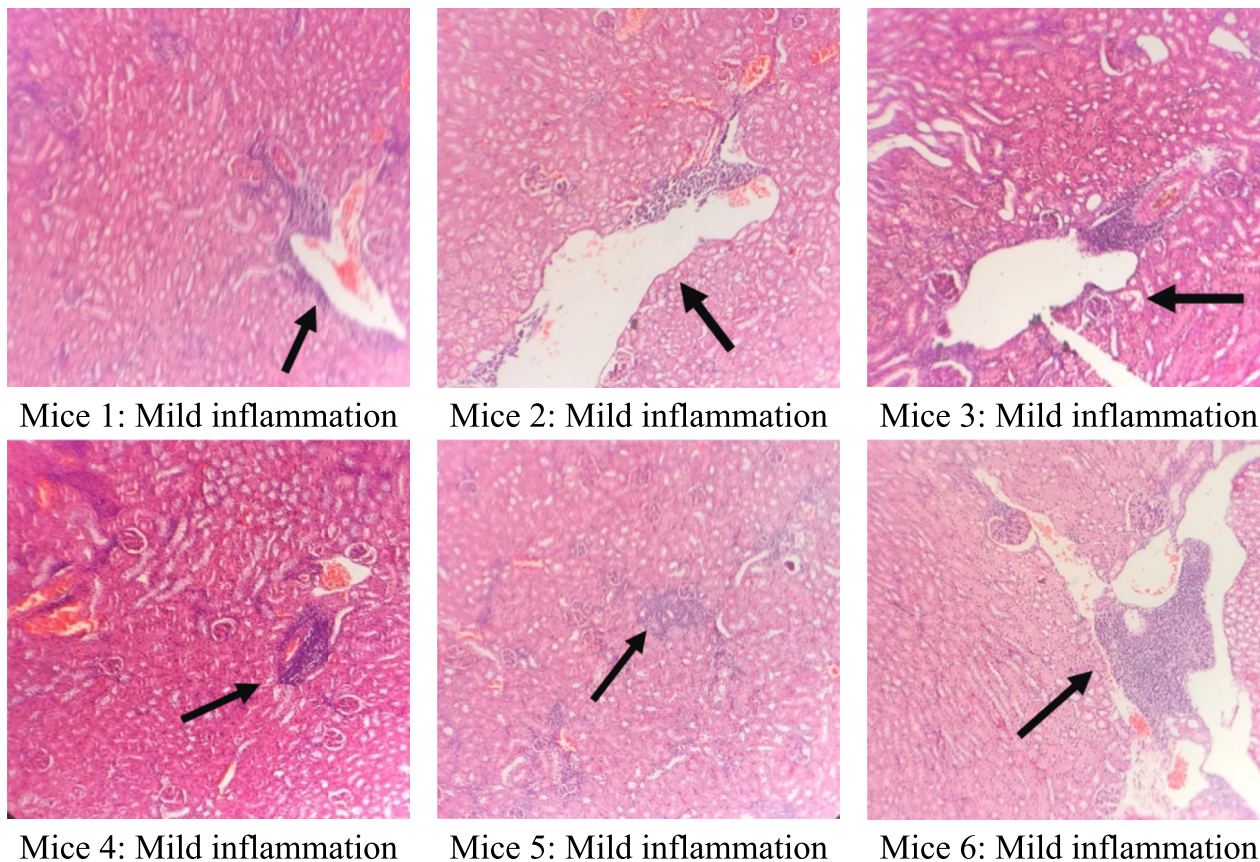


Fig. 6. Microstructure of mice kidney cells in the group treated with *C. flavescens* extract dose of 400 mg/kg.

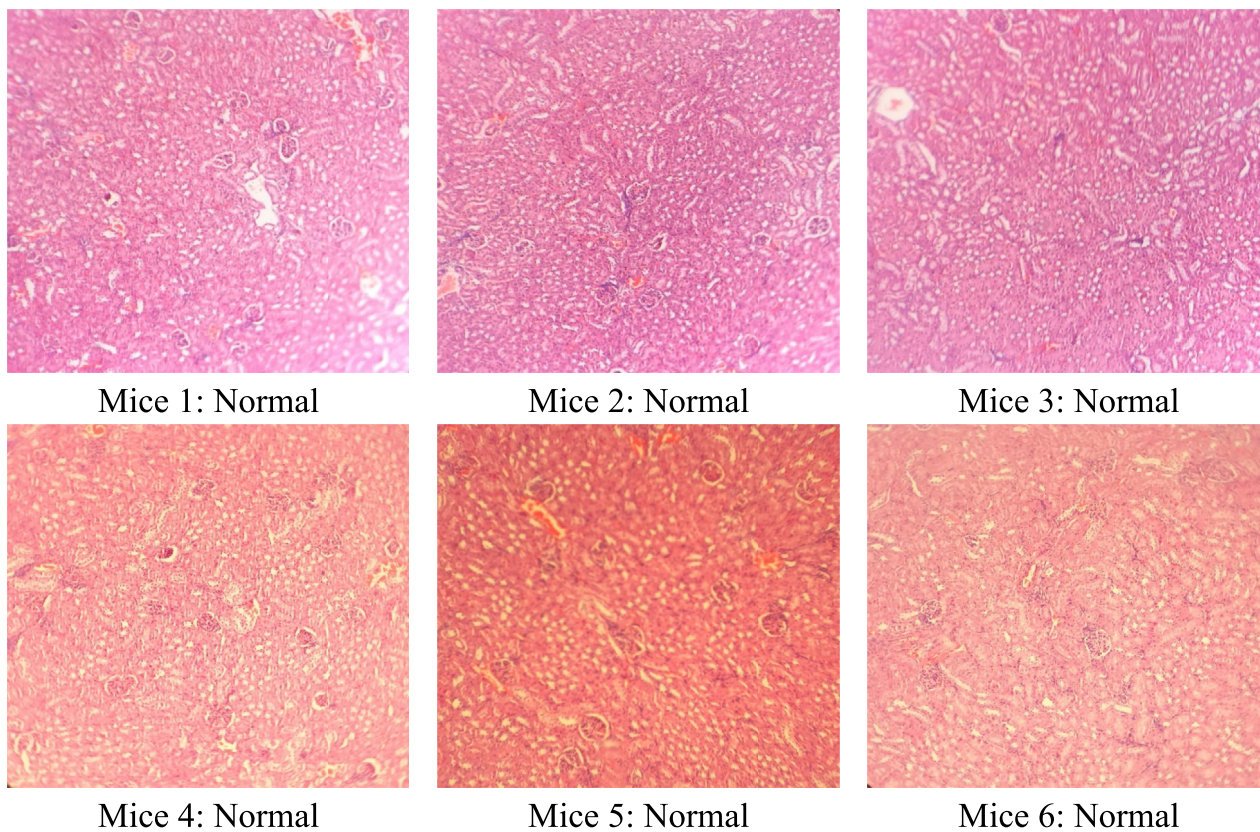


Fig. 7. Microstructure of mice kidney cells in the group treated with *C. flavescens* extract dose of 1200 mg/kg.

Because of their susceptibility to toxic compounds, hematological parameters must be evaluated when determining health status. In addition, blood profiling will often provide meaningful information about the body's response to injury and stress (Ezeja et al., 2014). It has been observed that increased white blood cell release is a biomarker of stress. However, it also aids in protecting the body against certain inflammatory conditions, infections, leukemias, and hemorrhages. In general, oral administration of *C. flavescens* extract to mice at doses of 400 mg/kg and 1200 mg/kg for 30 or 60 consecutive days did not cause any significant effects on hematopoietic function compared with the control mice. Thereby, it was shown that plant extracts did not cause toxic effects on the hematopoietic system.

The liver and kidney serve as source organs for xenobiotic activity, with the liver acting as the primary organ for xenobiotic biotransformation and the kidney acting as an excretory organ for xenobiotics (Unuofin et al., 2018). The serum concentration of bioidentical enzymes is a commonly used biochemical parameter to observe the toxic effects on the liver (J. T. Mukinda & Syce, 2007). Among them, AST and ALT, also known as transaminases, are sensitive indicators of hepatocellular injury, and their elevation is thought to occur due to hepatocellular necrosis, leading to their leakage into the blood during toxic liver injury (Ezhilarasan et al., 2012). The biochemical indicators related to the liver, such as AST, ALT, bilirubin, albumin, and cholesterol, showed no abnormal changes compared with the control group. Accumulation of cholesterol is one cause of insulin-mediated impairment of metabolism, which can contribute to type 2 diabetes (Ezeja et al., 2014). For the kidney, dysfunction in this part is usually determined through the change of urea and creatinine (James T. Mukinda & Eagles, 2010). The serum transaminases (ALT and AST), bilirubin, albumin, cholesterol in the liver, and the values of urea and creatinine in the kidney were not affected by the *C. flavescens* extract, which is a definite sign that it does not harm these organs (Tables 3 and 4). The extract from *C. flavescens* is expected to have significant potential in aiding the control of cardiovascular issues and providing support for patients with diabetes. This outcome is in line with earlier research showing that *C. flavescens* extract is a source that can be used to obtain polyphenolic compounds, which have been shown to help prevent type 2 diabetes (Nguyen et al., 2022; Pham et al., 2022; Fernandes et al., 2022). Histological evaluation was used as a benchmark to identify pathological changes in tissues and organs in test animals. The histological analysis of the liver and kidney in this study showed that the use of *C. flavescens* plant extract at doses of 400 and 1200 mg/kg affected the microstructure of the liver after 60 days of use. At the same time, the microstructure of the kidney was observed to have a slight effect when using the plant extract at a dose of 400 mg/kg for 60 days. In general, the change of microstructure in these organs is insignificant. These also support the confirmation of previous results of slight variations in hematological parameters and biochemical parameters of the liver and kidneys. Previous studies have reported that damage and increased liver and kidney biochemical parameters are related to oxidative stress (Ezhilarasan et al., 2012; Thangavelu et al., 2020). Oxidative stress is an imbalanced state caused by excessive free radicals that overwhelm endogenous antioxidant capacity, resulting in the oxidation of a biological macromolecule, such as enzymes, proteins, DNA, and lipids. Oxidative stress is essential to chronic degenerative diseases, including coronary heart disease, cancer, and ageing. Much evidence has been demonstrated for the close association of oxidative stress with the onset of various diseases in humans and laboratory animals (Ezhilarasan & Karthikeyan, 2016). The *C. flavescens* extract has been reported to have a potent antioxidant capacity, which has also been confirmed by ensuring biochemical parameters and minimizing abnormal changes in the body of experimental animals in this study

(Nguyen et al., 2020). Based on the results obtained, it can be seen that the use of *C. flavescens* has the potential to support the treatment of some health issues and does not significantly cause adverse changes to the quality of life of the experimental animals when used for an extended period.

5. Conclusion

The study on subacute and subchronic toxicity of *C. flavescens* extract revealed that repeated administration of this extract for 60 days, even at high doses (1200 mg/kg), did not result in any appreciable change in hematological parameters. There were no abnormal changes observed in the liver and kidney function indices. The histological structure of the liver changed after 60 days of treatment with *C. flavescens* at both doses of 400 mg/kg and 1200 mg/kg, whereas the histological structure of the kidneys changed at the 400 mg/kg dose compared to the control group. Overall, this study suggests that *C. flavescens* extract can be considered safe to use, but further studies are needed on renal effects with long-term use.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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