Toxicology Reports 12 (2024) 224–233

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Oral sub-chronic toxicity of fingerroot (*Boesenbergia rotunda*) rhizome extract formulation in Wistar rats

Pinnakarn Techapichetvanich [a], Supawit Tangpanithandee [b], Kittitach Supannapan [c], Supakit Wongwiwatthananukit [d], Leng Chee Chang [d], Phisit Khemawoot [b,*]

[a] *Program in Translational Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
[b] *Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakarn, Thailand*
[c] *Chao Phya Abhaibhubejhr Hospital Foundation, Prachinburi, Thailand*
[d] *The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI, USA*

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Boesenbergia rotunda
Cyclodextrin
Fingerroot
Sub-chronic toxicity
NOAEL

ABSTRACT

Background: Boesenbergia rotunda (fingerroot) rhizome extract contains two major bioactive components, panduratin A and pinostrobin. In our previous study, we found the anti-inflammatory effects of the fingerroot extract against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in golden Syrian hamsters. In the present study, we evaluated the sub-chronic toxicity of a fingerroot extract formulation over 90 consecutive days of oral administration.

Methods: We enhanced the water solubility of a fingerroot extract by formulating it with cyclodextrin, containing panduratin A (29% w/w) and pinostrobin (32% w/w). This formulation was administered to male and female Wistar rats at doses of 25, 50, or 100 mg/kg/day for a duration of 90 days. Additionally, two recovery groups, comprising a control group and a high-dose group, were designated for a 14-day observation period to assess the persistence and reversibility of potential adverse effects. Throughout the experiment, we performed clinical and health observations, followed by hematological testing, clinical biochemistry analysis, necropsy examination, and histopathological evaluation at the end of the experiment.

Results: The administration of the fingerroot extract formulation at doses of 25, 50, or 100 mg/kg/day did not result in mortality or clinical signs of toxicity. No clinically significant findings were associated with the oral administration of the fingerroot extract formulation.

Conclusion: The fingerroot extract formulation showed no serious adverse effects at doses up to 100 mg/kg/day in Wistar rats under the experimental condition. Consequently, the No Observed Adverse Effect Level (NOAEL) was considered to be 100 mg/kg/day. This finding contributes significance for future developments involving fingerroot extract in herbal medicinal products targeting chronic inflammation.

List of abbreviation: ACC, Acetyl-CoA carboxylase; ALB2, Albumin; ALP2S, Alkaline phosphatase; ALTL, Alanine aminotransferase; AMPK, Adenosine monophosphate-activated protein kinase; ASTL, Aspartate aminotransferase; BASO, Basophil; CHO2l, Cholesterol; Cl, Chloride; CREA2, Creatinine; C/EBPα, CCAAT/enhancer-binding protein alpha; EO, Eosinophil; GLO, Globulin; GSK3β, Glycogen synthase kinase 3β; HCT, Hematocrit; HDLC4, High-density lipoprotein; HGB, Hemoglobin; IL, Interleukin; JNK, c-Jun N-terminal kinase; K, Potassium; LDLC3, Low-density lipoprotein; LPS, Lipopolysaccharide; LYMPH, Lymphocyte; MAPK, Mitogen-activated protein kinases; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; MCV, Mean corpuscular volume; MONO, Monocyte; Na, Sodium; NF-kappaB, Nuclear factor kappaB; NO, Nitric oxide; NOAEL, No observed adverse effect level; NEUT, Neutrophil; OECD GLP 408, The Organization for Economic Co-operation and Development Guideline for;Testing of Chemicals 408; PGE_2, Prostaglandin E_2; PLT, Platelet; PPARγ, Peroxisome proliferator-activated receptor gamma; p38, Stress-activated protein kinase; RBC, Red blood cell count; SGLU3, Glucose; SIM-A9, Spontaneous immortalized microglia-A9; SREBP-1c, Sterol response element-binding protein-1c; TLR4/MD2 complex, The toll-like receptor 4/myeloid differentiation factor 2 complex; TNF-α, Tumor Necrosis Factor alpha; TP2, Total protein; TRIGL, Triglyceride; TSH, thyroid stimulating hormone; T3, triiodothyronine; T4, thyroxine; UA2, Uric acid; U-BUN, Blood urea nitrogen; WBC, White blood cell count.

* Correspondence to: Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bang Phli, Samut Prakarn 10540, Thailand.

E-mail address: phisit.khe@mahidol.ac.th (P. Khemawoot).

https://doi.org/10.1016/j.toxrep.2024.01.013
Received 8 December 2023; Received in revised form 22 January 2024; Accepted 28 January 2024
Available online 30 January 2024

1. Introduction

Boesenbergia rotunda Mansf. (Fig. 1A), a member of the Zingiberaceae family, commonly known as fingerroot or referred to as *Boesenbergia pandurata*, *Kaempferia pandurata*, is widely used in Southeast Asia for culinary and traditional medicinal purposes [1]. Research on the rhizome extracts of this plant (Fig. 1B) has revealed a diverse array of biological activities, including anti-inflammatory, antioxidant, antibacterial, anti-herpes simplex virus, and hepatoprotective effects [2–7]. The key phytochemicals identified in *B. rotunda* include alkaloids, flavonoids, essential oils, and phenolic compounds, with panduratin A and pinostrobin as predominant constituents [8,9].

Panduratin A (Fig. 1C), a cyclohexenyl chalcone compound abundant in fingerroot, possesses a molecular weight of 406.51 g/mol ($C_{26}H_{30}O_4$) and exhibits a wide range of beneficial effects, including anti-inflammatory, antioxidative, antimutagenic, antibacterial, anticancer, anti-allergy, and anti-obesity properties [10]. Pharmacokinetic studies in rats indicate that orally administered fingerroot extract

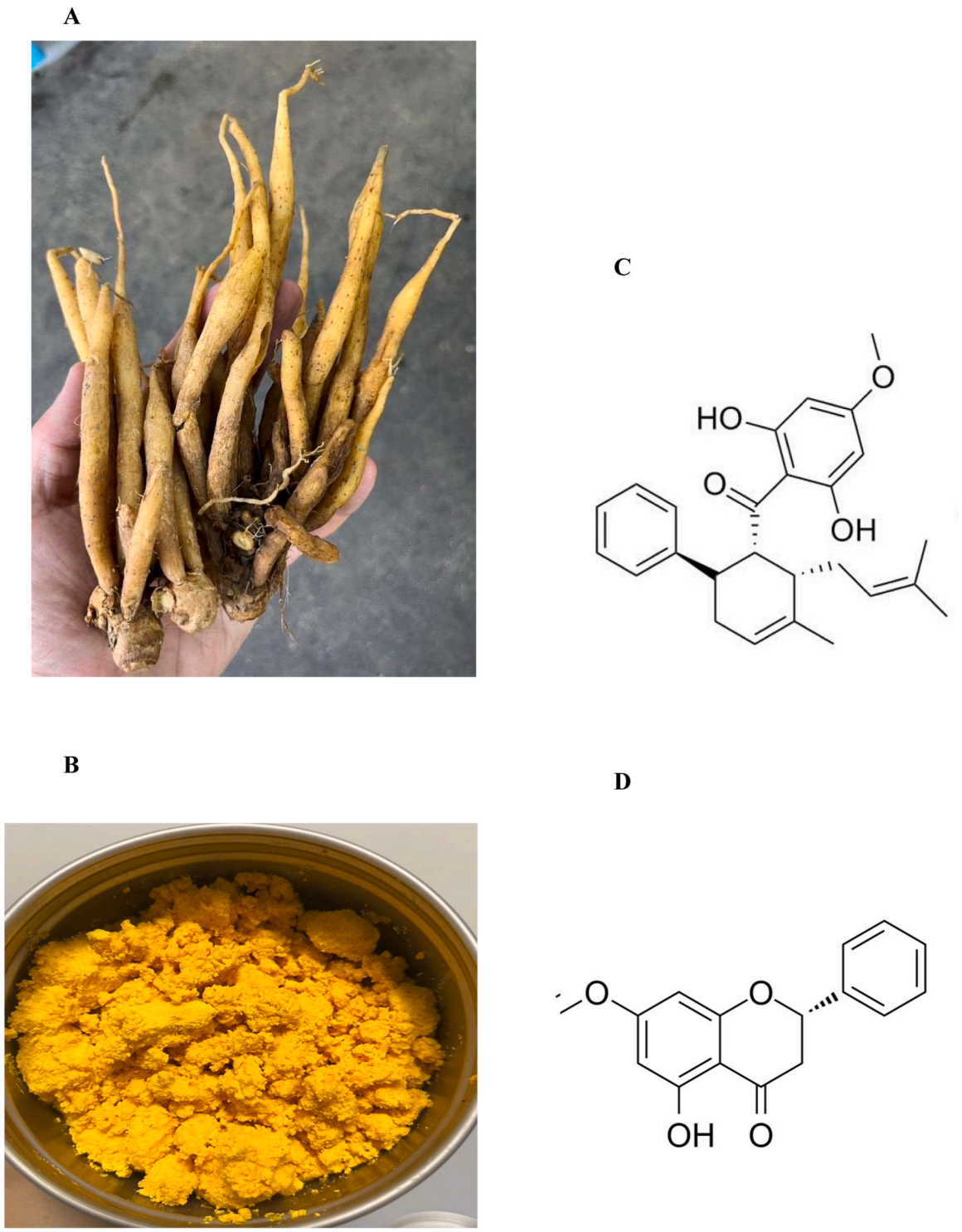

Fig. 1. (A) *Boesenbergia rotunda* (Roxb.) Schltr; (B) the physical appearance and formulation of fingerroot extract; (C) the chemical structures of panduratin A and (D) pinostrobin.

distributes panduratin A across various tissues, such as the skin, lung, heart, liver, spleen, kidney, and brain [11]. Interestingly, fingerroot extract and its isolated compound, panduratin A, have demonstrated significant inhibitory effects against the replication and infectivity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Vero E6 cells, with a favorable cytotoxicity profile [12]. However, the late application of fingerroot extract to SARS-CoV-2-infected hamsters resulted in limited efficacy in reducing viral output. Nonetheless, there is potential for fingerroot extract to alleviate lung inflammation during the late stages of infection [13]. Chronic inflammation caused by exaggerated and prolonged inflammatory responses might lead to cardiovascular disease, type 2 diabetes, rheumatoid arthritis, and cancers [14,15]. Fingerroot extract possesses cardioprotective effects by mitigating this inflammation [1]. An ethanolic extract of *B. rotunda* could inhibit the expression of Akt and nuclear factor kappa (NF)-κB p65 in the stomach and intestine of acetic acid–induced Wistar rats [16]. In addition, a bioactive compound from fingerroot was found to decrease the plasma level of interleukin 6 (IL-6) in a rat model of ulcers [17]. In the mouse macrophage RAW264.7 cells, panduratin A presented potent inhibitory activity against nitric oxide (NO) and anti-prostaglandin E_2 (PGE_2), with a half-maximal inhibitory concentration (IC_{50}) of 0.175 and 0.0195 μM, respectively [18]. It significantly decreased messenger RNA (mRNA) levels and the release of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-1β, and IL-6. Simultaneously, panduratin A increased the release of the anti-inflammatory cytokines IL-4 and IL-10, effectively attenuating the pro-inflammatory stage in neuroinflammatory diseases [19,20]. An *in vitro* study focused on the prevention of neuroinflammation-associated neurodegenerative diseases found that panduratin A counteracted lipopolysaccharide (LPS)-induced microglial activation in the spontaneous immortalized microglia-A9 (SIM-A9) cell line by reducing NO levels and pro-inflammatory cytokine production, and secretion [21]. Pinostrobin (Fig. 1**D**), known as 5-hydroxy-7-methoxyflavanone (molecular weight of 270.28 g/mol, $C_{16}H_{14}O_4$), also has documented pharmacological benefits, notably anticancer and antioxidant activities [22]. Recent studies highlight its role in mitigating inflammation and oxidative stress in cellular models [23,24]. For example, pinostrobin inhibited LPS-induced TNF-α and IL-1β expression in vitro and in vivo [24]. The authors found that pinostrobin exerted anti-inflammatory effects in LPS-stimulated RAW 264.7 macrophages and endotoxemia by binding to the Toll-like receptor 4/myeloid differentiation factor 2 (TLR4/MD2) complex. Moreover, pinostrobin noticeably attenuated the mortality of and abnormalities in LPS-microinjected zebrafish larvae [23].

Despite these plant extracts demonstrating pharmacological activities, it is crucial to evaluate their safety, particularly when considering their potential toxicity in vivo [25]. Toxicity studies in animals provide insight into potential human reactions, correlating efficacy with the safety profile. The benefits of toxicological assessment of plant extracts in animals include a controlled exposure period, the examination of various tissues for potential toxicity, and the determination of biological effects in different organs. Rodents are recommended for toxicological research to determine the safe doses for humans and the registration of new investigational drugs [26,27]. For example, a sub-chronic toxicity study revealed that oral administration of the ethanolic extract of *B. rotunda* at 60, 120, or 240 mg/kg/day in male rats for 60 days shows no signs of toxicity [28]. Similarly, the oral administration of pinostrobin and pinocembrin from *B. rotunda* at a dosage of 100 mg/kg/day for 7 days revealed no signs of toxicity in male Wistar rats [29]. However, these toxicity studies often overlook the percentages of active ingredients like panduratin A and pinostrobin; instead, they focus on general markers such as blood biochemistry and organ pathology.

The advancement in pharmacognosy research has improved percent yield of bioactive compounds from natural resources. In the case of our fingerroot extract, it contained panduratin A 29% w/w and pinostrobin 32% w/w which are approximately 10–20 folds larger than the natural occurrence in the rhizome of *B. rotunda*. In the regulatory point of view, these enriched extracts have very high percentages of bioactive compounds and need a general toxicity study to evaluate their safety profile before entering into clinical trials. Fingerroot extract and its bioactive constituents show limited water solubility, which reduces oral bioavailability and might mask its real toxicity. Therefore, we developed a formulation by mixing fingerroot extract with β-cyclodextrin at 1:2 ratio to improve water solubility. This formulation has been tested for oral pharmacokinetics in Beagles, improving tolerability in dogs after consecutive dosing for 7 days [30]. Panduratin A was detected in dog plasma up to 72 h after oral dosing. In addition, there were no adverse events or abnormalities in blood biochemistry and hematological parameters in dogs after receiving the formulation for 7 consecutive days. In this study, we examined the repeated dose toxicity of the fingerroot extract formulation in male and female rats over a 90-day period. The duration of this study aligns with the guidance provided by ICHM3 (R2) guidance, which recommends a study period ranging from 2 weeks to 6 months. This timeframe corresponds to the typical duration of treatment for long COVID syndrome. Doses were selected based on the efficacy of fingerroot extract in SARS-CoV-2-infected hamsters and the pharmacokinetic data in dogs. The information supports the development of phytopharmaceutical products from fingerroot extract as anti-inflammatory agents for chronic inflammation, especially long COVID, where treatment is limited.

2. Material and methods

2.1. Collection, identification, and preparation of plant material

The fingerroot extract was obtained via carbon dioxide supercritical extraction; it was a yellow semisolid and had a characteristic odor. The extract was provided by the Chao Phya Abhaibhubejhr Hospital Foundation under the Royal Patronage of H.R.H. Princess Bejraratanarajsuda (Prachinburi, Thailand). The extract lot number RD-001KEF contained panduratin A 29% (w/w) and pinostrobin 32% (w/w) as determined by liquid chromatography–mass spectrometry. The fingerroot extract was stored in a custodian room at 4 °C until it was formulated with β-cyclodextrin to improve water solubility. Each 100 mg of the fingerroot extract formulation contained 20 mg of fingerroot extract, 40 mg of cyclodextrin, and 40 mg of other diluents. The procedure used to develop the fingerroot extract formulation was described by Boonyarattanasoonthorn *et al.* [30].

2.2. Selection and maintenance of animals

One hundred Wistar rats (50 females and 50 males) obtained from the National Laboratory Animal Center, Mahidol University, Thailand, were used in the 90-day repeated-dose oral toxicity study. Six-week-old rats that weighed 159–187 g (males) or 137–164 g (females) were used. The animals were housed in plastic cages filled with corn cob at 22 ± 3 °C, a pressure of 34.3–62.6 Pa, a relative humidity of 30%− 70%, and a 12-hour photoperiod. The standard diet (082: Perfect Companion Group, Thailand) and reverse osmosis water were provided ad libitum. All animals were acclimatized to laboratory conditions for at least 5 days before the experiment started. The animals were weighed and randomly distributed into six groups (Table 1). Group 1 served as the vehicle control (distilled water) with 10 males and 10 females. For the treatment groups, 10 males and 10 females were administered fingerroot extract formulation at low dose (25 mg/kg/day), medium dose (50 mg/kg/day), and high dose (100 mg/kg/day). For the recovery groups, control-recovery (5 males and 5 females), and high dose-recovery (5 males and 5 females) were continuously observed for 14 days post-study completion. The effective dose range for acute SARS-COV2 infection was determined to be 300–1000 mg/kg/day for 7 days. Calculations indicated a similar total exposure of 10–100 mg/kg/day for 90 days, which was an appropriate concentration for chronic inflammation. Thus, doses of 25, 50, and 100 mg/kg/day were selected for this study. The mean weight

Table 1
The experimental group of Wistar rats receiving fingerroot extract formulation 25, 50, 100 mg/kg/day PO for 90 days.

Group	Animal No.	Sex	Dose Levels
		Main	
1	1-10	Male	Control
	11-20	Female	(Distilled water)
2	21-30	Male	Low dose
	31-40	Female	(25 mg/kg body weight)
3	41-50	Male	Medium dose
	51-60	Female	(50 mg/kg body weight)
4	61-70	Male	High dose
	71-80	Female	(100 mg/kg body weight)
		Recovery	
5	81-85	Male	Control-recovery
	86-90	Female	(Distilled water)
6	91-95	Male	High dose -recovery
	96-100	Female	(100 mg/kg body weight)

difference between each group was not more than 20%. The fingerroot extract formulation was calculated, weighed, and dissolved using distilled water at three dose levels: 25, 50, and 100 mg/kg body weight. The fingerroot extract formulation used for dosing was freshly prepared every day immediately before the administration. The oral administration was performed by passing the gavage needle into the esophagus in a straight line to the stomach once a day for a period of 90 days.

2.3. Ethics statement

This study adhered to the guidelines for the care and use of laboratory animals (Institute of Laboratory Animal Resources, NIH publication number #85–23, revised 2011). The study protocol was in compliance with OECD GLP 408 [31], holding GLP certification number 23/58 and approval date on Jan 2, 2019. The study was approved by the National Laboratory Animal Center Animal Care and Use Committee, Mahidol University, Thailand (NLAC-ACUC No. RA2021–40) on September 23, 2021. All animal experiments complied with the ARRIVE guidelines and Animals for Scientific Purposes Act (Thailand), A.D. 2015.

2.4. Clinical observation and health examination

The daily observation focused on changes in general clinical signs at a similar time and in a standard area. These observations were made outside the cage. Individual body weights were recorded once during the acclimatization period and once a week until the day of necropsy. Feed and drinking water consumption were measured once during the acclimatization period and then daily after the first day of dosing. The following clinical signs of toxicity were examined once a week: health examinations including changes in skin, fur/coat, eyes, and mucous membrane; the occurrence of secretions and excretions; autonomic activity (lacrimation, piloerection, pupil size, and the respiratory pattern); changes in gait, posture, and response to handing; the presence of clonic and tonic movements, stereotyped/bizarre behavior (excessive grooming, repetitive cycling, self-mutilation, and walking backwards); and neurological examinations (auditory, visual, proprioception, motor activity assessment, and fore-limb and hind-limb grip strength test). The ophthalmological status of all rats was examined using an ophthalmoscope on day0 before the administration of the test compound and on day90 upon completion of the study. Various ophthalmologic organs, including eyelids, conjunctiva, cornea, iris, lens, and fundus, were examined.

2.5. Clinical biochemistry and hematological testing

Blood samples were collected from the posterior vena cava. Whole blood samples were separated into two tubes for clinical biochemistry and hematological analyses. Clinical biochemistry parameters including sodium (Na), potassium (K), chloride (Cl), glucose (SGLU3), cholesterol (CHO2l), triglyceride (TRIGL), uric acid (UA2), blood urea nitrogen (U-BUN), creatinine (CREA2), total protein (TP2), albumin (ALB2), globulin (GLO), high-density lipoprotein (HDLC4), low-density lipoprotein (LDLC3), alanine aminotransferase (ALTL), aspartate aminotransferase (ASTL), alkaline phosphatase (ALP2S), triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) were examined in serum obtained after centrifugation of total blood without anticoagulant using a Cobas C311 automated blood analyzer (Roche, Switzerland). Hematological parameters including the red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), the white blood cell count (WBC), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EO), and basophils (BASO) were analyzed with an automated analyzer (IDEXX Procyte DX, USA).

2.6. Anatomical pathology

After euthanizing the animals with carbon dioxide inhalation, organs were removed for pathological examination. At the time of necropsy, the stage of the estrous cycle (metestrus, diestrus, and proestrus) of all female animals was determined by taking vaginal smears. Organs (liver, kidney, heart, adrenal gland, brain, testes, prostate glands, epididymis, ovaries and oviduct, uterus, spleen, thymus, thyroid and parathyroid glands, and pituitary gland) were removed, weighed (all the paired organs were weighed separately), and preserved in 10% (v/v) neutral-buffered formalin in plastic bags. The weights of these organs were converted to relative organ weights (organ-to-body weight ratios). For histopathological analysis, selected organs (liver, kidney, heart, lung, spleen, thyroid and parathyroid glands, stomach, small intestine, and large intestine) were removed and preserved in 10% (v/v) neutral-buffered formalin to prepare tissue sections. The pathologist performed the histopathological examination and immediately recorded the findings. The diagnostic terms and glossary are based on the International Harmonization of Nomenclature and Diagnostic Criteria. Lesion scoring was classified into five levels namely absent, minimal, mild, moderate, and severe, correlating to 0, + 1, + 2, + 3, and + 4, respectively, and was generally applied semi-quantitatively in direct proportion to the number of foci or the area of lesions.

2.7. Statistical analysis

The quantitative results are expressed as the mean ± standard deviation. All statistical analyses were conducted using SPSS Statistics version 18.0.0 (SPSS Inc. USA). A p-value < 0.05 was considered to be statistically significant. Normality and homogeneity of variances were assessed through the Kolmogorov-Smirnov test and Levene's test, respectively. For parametric statistics, homogenous data were compared between the vehicle control group and each treatment group using one-way analysis of variance (ANOVA), followed by the two-sided Dunnett's test. Heterogeneous data were compared using one-way ANOVA followed by the two-sided Dunnett's T3 test. Non-parametric statistics involved comparing data between the vehicle control group and each treatment group using the Mann-Whitney U test. The quantitative results for the recovery group are expressed as an average ± standard deviation. Levene's test was utilized to assess homogeneity of variances, and a Student's t-test for equality of means was applied to compare the vehicle control recovery group with the high-dose recovery group.

3. Results

3.1. Clinical observation and health examination

Throughout the 90-day study, none of the rats showed abnormalities or signs of toxicity. There were isolated incidents of minor hair loss and

skin scaling in a few female rats. The rats consistently gained weight across all groups, with no significant differences compared with the control group (Figs. 2A and B). Notably, food and water consumption varied in some test groups compared with the control group, as evidenced by a significant decrease in food consumption for both sexes in the low-dose group (Figs. 2C and D) and an increase in water consumption in the high-dose group (Figs. 2E and F).

Neurological and motor assessments revealed no neurological issues among the rats. The male rats in some test groups showed reduced motor activity, particularly at weeks 11 and 13 (Fig. 3A); this tendency was not observed in female rats (Fig. 3B). Both fore-limb (Figs. 3C and D) and hind-limb (Figs. 3E and F) grip strength remained generally unaffected, except for a noted decrease in male hind-limb strength in the high-dose group at week 12 (Fig. 3E).

3.2. Clinical biochemistry and hematological parameters

The biochemistry parameters for all rats fell within the normal ranges for healthy rats. While certain parameters, such as creatinine and high-density lipoprotein, showed statistically significant differences only in male rats, these variations remained within the established normal values for healthy rats. Thus, they are deemed clinically insignificant, as shown in Table 2. Hematological parameters also exhibited some statistically significant differences, yet they remained within the normal ranges for healthy rats. This included an elevated white blood cell count in the medium and high dose groups of both sexes. All of these observation changes are considered clinically insignificant. The comprehensive presentation of the hematological results is illustrated in Table 3.

3.3. Anatomical pathology

Organ weights were generally consistent across the groups, with only a few exceptions in certain groups. Notably, in the high dose group, a significantly lower weight in the left adrenal gland of male rats was observed compared to the control group. However, this particular finding was not presented in the recovery group or among female rats in all groups, suggesting a likely coincidental occurrence. Additionally, an increase in the weight of the left thyroid and parathyroid glands in the high dose recovery group among male rats was also considered coincidental, as it was not found in all female groups or the other male treatment groups (Table 4). The estrus cycle evaluations revealed no abnormal cellular types (Supplementary Table 1). There were macroscopic and microscopic findings such as thymus hemorrhage and fluid retention, but these were not consistently linked to the test compound. Thymus hemorrhage was observed across various groups, including the control group (n = 3/10), low dose (n = 2/10), medium dose (n = 3/10), high dose (n = 3/10), recovery control (n = 1/5), and recovery high dose (n = 1/5). It was relatively rare in female rats, occurring only in the low dose group (n = 2/10). Given its occurrence across all groups, including controls, thymus hemorrhage is considered coincidental. Similarly, fluid retention in the uterus, observed in the control (n = 2/10), low dose (n = 2/10), high dose (n = 4/10), and recovery high dose (n = 1/5) groups, also appears coincidental, as it was not consistently found across treatment groups. Observations of the kidney and liver suggested no significant treatment-related changes. In liver observations, a minimal periportal and macrovesicular fatty change are found in only one male rat from the high dose group, and this phenomenon was not observed in female rats. In kidney observations, hyaline casts were noted at minimal to mild levels in male rats from the control (n = 1/10) and high dose groups (n = 2/10), and similarly in both control (n = 1/5) and high dose recovery groups (n = 1/5). Tubular basophilia was minimally observed in one male from the high dose group and one female from the control group. These findings are considered coincidental and not related to the treatment.

4. Discussion

The major problem of conventional anti-inflammatory agents is the safety profile after long-term use. The most common adverse events related to corticosteroid use are osteoporosis, immunosuppression, and metabolic/endocrine disorders [32]. Similarly, other widely used anti-inflammatory medications, such as non-steroidal anti-inflammatory drugs, have been linked to adverse events like stomach ulcers, renal dysfunction, and cardiovascular issues [33]. Therefore, new anti-inflammatory agents with a favorable safety profile for chronic use is an urgent need to treat long-term inflammatory diseases. Recently, the coronavirus disease 2019 (COVID-19) pandemic, caused by

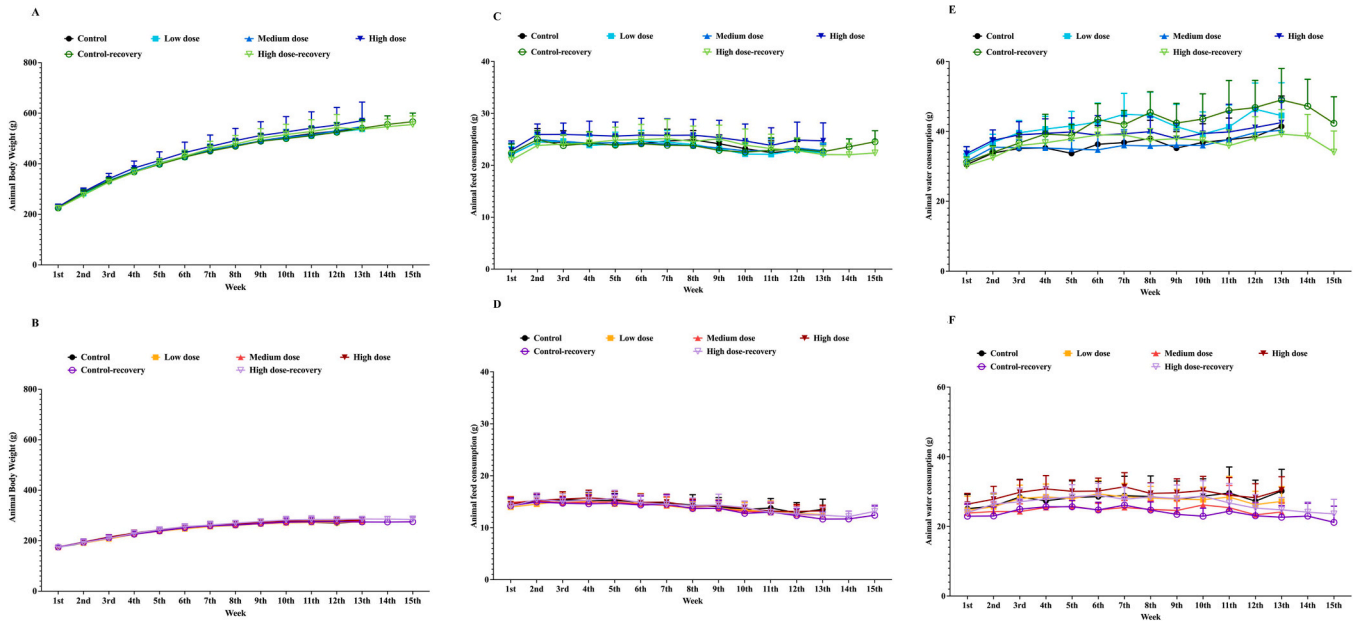

Fig. 2. The effects of the fingerroot extract formulation on the body weight of (A) male and (B) female rats, feed consumption of (C) male and (D) female rats, and drinking water consumption of (E) male and (F) female rats. The data are presented as the mean ± standard deviation (N = 10 per group). * $p < 0.05$ compared with the control group.

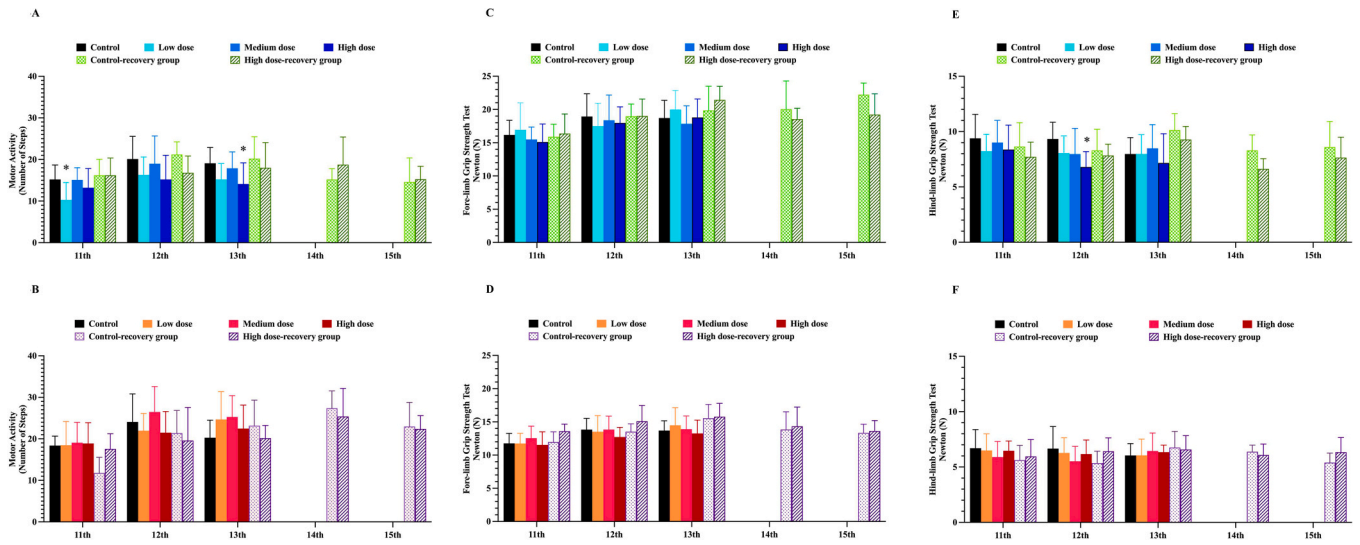

Fig. 3. The effects of the fingerroot extract formulation on the motor activity of (A) male and (B) female rats, the fore-limb grip strength of (C) male and (D) female rats, and the hind-limb grip strength of (E) male and (F) female rats. The data are presented as the mean ± standard deviation (N = 10). * $p < 0.05$ compared with the control group.

Table 2
The clinical biochemistry analysis of Wistar rats receiving fingerroot extract formulation 25, 50, 100 mg/kg/day PO for 90 days.

Sex	Parameter	Control	Main group			Recovery Group	
			Low	Medium	High	Control	High Dose
Male	SGLU3 (mg/dL)	328.5 ± 12.32	326.7 ± 32.95	308.5 ± 25.04	327.2 ± 24.64	348.2 ± 26.41	327.6 ± 48.42
	U-BUN (mg/dL)	18.9 ± 1.59	20.0 ± 1.15	20.8 ± 1.61[a]	19.9 ± 1.44	20.7 ± 0.61	20.8 ± 1.22
	CREA2 (mg/dL)	0.41 ± 0.02	0.39 ± 0.04	0.37 ± 0.04[a]	0.36 ± 0.02[a]	0.39 ± 0.02	0.37 ± 0.01
	UA2 (mg/dL)	6.4 ± 0.36	6.3 ± 0.56	5.5 ± 0.63[a]	6.1 ± 0.51	6.5 ± 0.47	5.8 ± 1.00
	CHO2I (mg/dL)	78.5 ± 8.11	83.3 ± 15.15	82.9 ± 11.33	83.2 ± 6.85	77.6 ± 10.31	83.9 ± 11.23
	TRIGL (mg/dL)	86.9 ± 14.13	88.4 ± 12.00	83.7 ± 14.67	111.8 ± 53.03	85.3 ± 17.82	90.4 ± 26.55
	LDLC3 (mg/dL)	10.8 ± 3.83	13.1 ± 5.60	14.0 ± 4.41	10.5 ± 2.27	10.8 ± 2.91	13.1 ± 5.05
	ASTL (U/L)	103.0 ± 20.71	89.8 ± 12.18	91.3 ± 14.20	93.1 ± 14.67	116.4 ± 24.23	100.4 ± 13.74
	ALTL (U/L)	70.8 ± 33.78	57.0 ± 12.92	61.0 ± 15.32	58.0 ± 11.46	99.0 ± 33.12	84.0 ± 29.99
	ALP2S (U/L)	85 ± 12.38	95 ± 7.64	80 ± 11.45	91 ± 19.14	86 ± 5.32	79 ± 7.02
	TP2 (g/dL)	6.92 ± 0.21	7.08 ± 0.16	7.05 ± 0.18	7.07 ± 0.23	6.91 ± 0.11	6.99 ± 0.01
	ALB2 (g/dL)	4.92 ± 0.18	5.08 ± 0.11	5.02 ± 0.15	5.05 ± 0.15	4.89 ± 0.16	4.97 ± 0.11
	HDLC4 (mg/dL)	56.6 ± 5.87	65.1 ± 10.72[a]	63.3 ± 7.38[a]	62.4 ± 6.33[a]	59.9 ± 8.01	64.8 ± 7.35
	Na (mmol/L)	147 ± 1.14	147 ± 0.67	146 ± 1.16	149 ± 0.99[a]	146 ± 2.68	148 ± 1.73
	K (mmol/L)	9.61 ± 0.33	9.28 ± 0.55	9.12 ± 0.44	9.21 ± 0.63	9.74 ± 2.10	9.24 ± 0.69
	Cl (mmol/L)	102.1 ± 1.15	101.9 ± 0.96	100.2 ± 1.37[a]	101.8 ± 1.34	101.3 ± 0.93	101.9 ± 1.32
	GLO (mg/dL)	2.01 ± 0.12	2.01 ± 0.10	2.03 ± 0.07	2.02 ± 0.15	2.02 ± 0.12	2.02 ± 0.10
	T3 (ng/dL)	59.25 ± 15.80	60.25 ± 17.04	57.00 ± 19.03	64.75 ± 5.80	53.00 ± 10.23	65.25 ± 5.38
	T4 (μg/dL)	2.52 ± 0.30	2.64 ± 0.69	3.10 ± 0.36	2.81 ± 0.55	2.65 ± 0.34	2.93 ± 0.48
	TSH (μIU/mL)	0.26 ± 0.26	0.27 ± 0.19	1.26 ± 0.83[a]	0.85 ± 0.36	0.17 ± 0.14	0.28 ± 0.16
Female	SGLU3 (mg/dL)	177.4 ± 40.03	228.4 ± 45.73	193.8 ± 64.29	225.4 ± 45.37	170.3 ± 40.98	153.9 ± 56.15
	U-BUN (mg/dL)	17.8 ± 1.41	19.0 ± 2.15	19.7 ± 1.64	18.1 ± 2.44	20.7 ± 3.20	18.3 ± 2.49
	CREA2 (mg/dL)	0.40 ± 0.03	0.40 ± 0.02	0.42 ± 0.04	0.39 ± 0.04	0.44 ± 0.06	0.41 ± 0.03
	UA2 (mg/dL)	3.8 ± 0.40	4.0 ± 0.35	3.7 ± 0.49	4.1 ± 0.28	3.8 ± 0.49	3.5 ± 0.15
	CHO2I (mg/dL)	101.8 ± 13.39	86.3 ± 12.85[a]	94.6 ± 7.59	92.1 ± 13.23	97.9 ± 17.93	94.5 ± 18.59
	TRIGL (mg/dL)	60.0 ± 11.25	63.1 ± 14.06	59.4 ± 17.19	58.0 ± 10.21	71.1 ± 22.49	66.6 ± 23.43
	LDLC3 (mg/dL)	10.6 ± 3.29	8.2 ± 2.35	10.1 ± 2.15	8.6 ± 2.88	8.6 ± 3.02	9.8 ± 4.31
	ASTL (U/L)	87.4 ± 7.82	77.1 ± 6.71[a]	74.9 ± 7.58[a]	80.7 ± 7.18	73.1 ± 8.87	79.1 ± 4.63
	ALTL (U/L)	44.1 ± 10.04	38.1 ± 8.70	35.0 ± 4.86	37.5 ± 3.27	36.6 ± 5.69	35.6 ± 5.32
	ALP2S (U/L)	44 ± 4.69	45 ± 2.82	43 ± 4.03	43 ± 3.68	38 ± 5.79	40 ± 2.97
	TP2 (g/dL)	7.23 ± 0.13	7.09 ± 0.22	7.17 ± 0.28	7.17 ± 0.15	6.96 ± 0.22	7.20 ± 0.26
	ALB2 (g/dL)	5.45 ± 0.12	5.39 ± 0.19	5.38 ± 0.22	5.38 ± 0.12	5.33 ± 0.18	5.40 ± 0.22
	HDLC4 (mg/dL)	81.1 ± 9.22	73.4 ± 9.43	80.3 ± 5.35	77.0 ± 9.81	73.2 ± 13.24	77.8 ± 11.32
	Na (mmol/L)	146 ± 1.49	147 ± 1.27	147 ± 1.25	149 ± 1.51[a]	146 ± 1.52	147 ± 1.41
	K (m mol/L)	10.14 ± 0.75	9.60 ± 0.83	9.50 ± 0.60	9.62 ± 0.85	9.60 ± 0.62	10.00 ± 1.43
	Cl (mmol/L)	104.9 ± 1.77	105.3 ± 1.55	104.4 ± 1.16	105.0 ± 1.61	105.52 ± 1.05	104.30 ± 1.00
	GLO (mg/dL)	1.78 ± 0.07	1.70 ± 0.11	1.79 ± 0.16	1.79 ± 0.11	1.64 ± 0.06	1.80 ± 0.17
	T3 (ng/dL)	80.50 ± 11.09	77.25 ± 11.44	86.00 ± 11.78	103.00 ± 16.85	81.50 ± 17.94	109.75 ± 10.40
	T4 (μg/dL)	2.73 ± 0.41	2.81 ± 0.24	2.93 ± 0.44	2.47 ± 0.65	2.83 ± 0.93	3.36 ± 0.72
	TSH (μIU/mL)	0.20 ± 0.09	0.17 ± 0.05	0.30 ± 0.16	0.31 ± 0.14	0.26 ± 0.15	0.30 ± 0.12

bThe average is statistically significant difference at the 0.05 levels of control-recovery group.
Values are average ± standard deviation (n = 10, per group for male and female rats)
[a] The average is statistically significant difference at the *0.*05 levels of control group.

Table 3
Hematological analysis of Wistar rats receiving fingerroot extract formulation 25, 50, 100 mg/kg/day PO for 90 days.

Sex	Parameter	Control	Main group			Recovery group	
			Low	Medium	High	Control	High Dose
Male	RBC (M/μL)	9.59 ± 0.33	9.88 ± 0.20[a]	9.67 ± 0.20	9.73 ± 0.24	9.44 ± 0.34	9.72 ± 0.19
	HGB (g/dL)	17.0 ± 0.45	17.4 ± 0.30[a]	17.1 ± 0.30	17.0 ± 0.40	17.0 ± 0.65	17.3 ± 0.19
	HCT (%)	52.7 ± 1.57	54.7 ± 1.22[a]	53.5 ± 0.91	53.2 ± 1.48	53.2 ± 2.33	54.1 ± 0.64
	MCV (fL)	55.0 ± 1.65	55.4 ± 1.12	55.4 ± 0.47	54.7 ± 1.16	56.3 ± 0.75	55.7 ± 0.56
	MCH (pg)	17.7 ± 0.41	17.6 ± 0.34	17.6 ± 0.18	17.5 ± 0.36	18.0 ± 0.19	17.8 ± 0.17
	MCHC (g/dL)	32.2 ± 0.28	31.9 ± 0.34[a]	31.9 ± 0.20[a]	31.9 ± 0.21[a]	32.0 ± 0.26	31.9 ± 0.05
	PLT (K/μL)	693 ± 61.73	694 ± 45.83	700 ± 86.29	702 ± 36.94	669 ± 43.56	661 ± 26.78
	WBC (K/μL)	6.80 ± 1.26	6.57 ± 0.61	8.05 ± 0.68[a]	7.74 ± 1.01[a]	6.68 ± 0.86	6.72 ± 1.50
	NEUT (%)	11.3 ± 1.33	12.9 ± 2.25	11.5 ± 2.76	10.4 ± 1.86	12.5 ± 1.77	10.8 ± 2.65
	LYMPH (%)	83.4 ± 1.38	81.3 ± 1.99	83.4 ± 3.04	85.0 ± 2.82	80.1 ± 2.26	84.0 ± 1.98[b]
	MONO (%)	4.3 ± 0.70	4.6 ± 1.09	4.2 ± 1.02	3.7 ± 0.71	6.0 ± 1.55	4.3 ± 1.29
	EO (%)	0.8 ± 0.22	0.8 ± 0.20	0.7 ± 0.18	0.8 ± 0.45	1.2 ± 0.43	0.7 ± 0.22
	BASO (%)	0.2 ± 0.21	0.4 ± 0.17	0.1 ± 0.14	0.1 ± 0.17	0.2 ± 0.21	0.3 ± 0.22
Female	RBC (M/μL)	9.39 ± 0.42	9.11 ± 0.43	9.26 ± 0.36	9.16 ± 0.50	9.24 ± 0.32	9.62 ± 0.53
	HGB (g/dL)	17.4 ± 0.79	17.2 ± 0.56	17.4 ± 0.72	17.1 ± 1.01	17.3 ± 0.82	18.1 ± 1.48
	HCT (%)	53.4 ± 2.36	53.2 ± 2.06	53.9 ± 2.43	52.8 ± 3.58	53.4 ± 2.98	56.4 ± 5.10
	MCV (fL)	56.9 ± 0.99	58.4 ± 0.95[a]	58.2 ± 0.97[a]	57.6 ± 1.67	57.8 ± 1.39	58.5 ± 2.17
	MCH (pg)	18.5 ± 0.31	18.9 ± 0.43	18.7 ± 0.32	18.7 ± 0.50	18.7 ± 0.32	18.8 ± 0.58
	MCHC (g/dL)	32.6 ± 0.19	32.4 ± 0.30	32.2 ± 0.28[a]	32.5 ± 0.34	32.3 ± 0.31	32.1 ± 0.36
	PLT (K/μL)	743 ± 66.14	685 ± 80.68	705 ± 69.03	719 ± 59.07	778 ± 57.89	767 ± 96.55
	WBC (K/μL)	3.76 ± 0.92	4.37 ± 0.78	5.63 ± 0.87[a]	4.97 ± 0.98[a]	4.60 ± 1.08	5.31 ± 0.99
	NEUT (%)	11.2 ± 2.97	10.6 ± 2.35	9.0 ± 2.48	8.8 ± 3.96	9.4 ± 2.19	10.1 ± 1.25
	LYMPH (%)	83.7 ± 3.16	83.8 ± 2.34	86.1 ± 3.00	85.2 ± 5.02	83.6 ± 1.59	83.4 ± 1.94
	MONO (%)	4.2 ± 0.72	4.8 ± 0.91	4.3 ± 1.03	5.4 ± 1.28[a]	6.0 ± 1.55	5.7 ± 1.02
	EO (%)	0.7 ± 0.32	0.6 ± 0.23	0.4 ± 0.13[a]	0.4 ± 0.16[a]	0.8 ± 0.33	0.6 ± 0.22
	BASO (%)	0.2 ± 0.19	0.2 ± 0.15	0.2 ± 0.17	0.2 ± 0.24	0.2 ± 0.05	0.3 ± 0.10

b The average is statistically significant difference at the 0.05 levels of control-recovery group.Table 4. Animal organ weight (g) per 100 g body weight of Wistar rats receiving fingerroot extract formulation 25, 50, 100 mg/kg/day PO for 90 days.
Values are average ± standard deviation (n = 10, per group for male and female rats)
[a] The average is statistically significant difference at the *0.*05 levels of control group.

SARS-CoV-2, has led to a large number of severe acute respiratory tract infections with long-term systemic inflammation, commonly referred to as long COVID, affecting approximately 30%− 50% of infected individuals [34]. Patients with long COVID may have underlying diseases before the viral infection and require additional medicines to manage their condition. This can lead to polypharmacy, increasing the risk of potential drug–drug interactions and adverse drug events [35]. Therefore, the development of alternative medicines derived from commonly used natural ingredients with an acceptable safety profile might become a strategic approach for managing chronic inflammation. Such an approach minimizes the risk of adverse events, especially in patients with long COVID. Prior to the present study, a fingerroot extract formulation was developed and evaluated for its efficacy in SARS-CoV-2 infected hamsters, along with pharmacokinetics in healthy dogs [30]. Panduratin A has antiviral and anti-inflammatory activities and pinostrobin exerts good anti-inflammatory activity, but they have limited water solubility [8,12]. The fingerroot extract, which inherently possesses limited water solubility due to its essential oils, was formulated through the incorporation of β-cyclodextrin in a 1:2 ratio. This resulted in the creation of the Fingerroot Extract Formulation, significantly enhancing its water solubility. In our study, we prepared a solution by dissolving this powder formulation, which contains β-cyclodextrin, in distilled water for rodent administration. This improved water solubility would make it easier to prepare and administer formulations that contain bioactive compounds with low water solubility.

In this toxicity study, we evaluated the sub-chronic oral toxicity of the fingerroot extract formulation in Wistar rats over a 90-day period by adhering to OECD GLP 408 [31]. Male and female rats, across the three dosage groups (25, 50, 100 mg/kg/day), exhibited no clinical symptoms of toxicity, morbidity, or mortality. This absence of adverse effects extended to the ophthalmological status and neurological signs. We deemed isolated instances of physical health markers like alopecia and scaling skin to be unrelated to the formulation. During toxicological testing, an alteration in body weight can be one of the indicators of the adverse effects of the test formulation. Moreover, food and water consumption are critical to the physiological status of animals and the growth rate of experimental animals [36]. While weight gain was consistent and unaffected across all groups, there were notable differences in feed and water consumption in some of the test groups. However, these variations were transient and showed no correlation with the fingerroot extract dosage, suggesting no impact on overall animal health. The grip strength test, a vital measure of muscular and neurobehavioral health, revealed no significant differences in fore-limb strength. However, there were certain variations in motor activity and hind-limb grip strength, particularly in male rats. Although these differences were statistically significant, they were not dose dependent and were transient, indicating a lack of neurotoxic effects from the tested fingerroot extract formulation.

Blood biochemistry analysis served as a useful method for assessing systemic biochemical changes following exposure to xenobiotics or test compounds. Statistically significant decrease in serum creatinine levels in male rats was observed, particularly in the medium- and high-dose groups. Liver health indicators showed statistically significant decrease in ASTL levels for most treatment groups of female rats but not in male rats. However, these phenomena were statistically significant but lacked clinical importance. Therefore, these changes were considered coincidental. It's worth noting that other researchers have indicated potential hepatoprotective effects of similar compounds, suggesting the need for further investigation in this area [9,37,38]. Our results show a significant increase in high-density lipoprotein across all treatment groups (low, medium, high) in male rats compared to the control, while this change did not occur in female rats. Despite the statistical significance, the observed change falls within the normal range for healthy rats. Therefore, this change is considered clinically insignificant. Hematological analysis revealed a statistically significant increase in white blood cells in the medium and high dose groups for both males and females compared with the control. However, as these changes fell within the normal range for healthy rats, further

Table 4
Animal organ weight (g) per 100 g body weight of Wistar rats receiving fingerroot extract formulation 25, 50, 100 mg/kg/day PO for 90 days.

Sex	Organ	Control	Main group			Recovery group	
			Low	Medium	High	Control	High Dose
Male	Liver	2.5986 ± 0.12	2.5303 ± 0.16	2.6397 ± 0.14	2.7364 ± 0.17	2.5515 ± 0.11	2.4907 ± 0.06
	Kidney Rt.	0.2584 ± 0.02	0.2584 ± 0.02	0.2573 ± 0.02	0.2567 ± 0.02	0.2582 ± 0.01	0.2402 ± 0.01[b]
	Kidney Lt.	0.2522 ± 0.02	0.2467 ± 0.03	0.2459 ± 0.02	0.2446 ± 0.02	0.2484 ± 0.01	0.2414 ± 0.01
	Heart	0.3000 ± 0.02	0.2986 ± 0.02	0.3055 ± 0.01	0.2961 ± 0.01	0.2856 ± 0.02	0.3095 ± 0.02
	Spleen	0.1757 ± 0.01	0.1727 ± 0.02	0.1780 ± 0.01	0.1835 ± 0.02	0.1793 ± 0.01	0.1711 ± 0.02
	Brain	0.4202 ± 0.02	0.4234 ± 0.03	0.4220 ± 0.02	0.3994 ± 0.05	0.4061 ± 0.03	0.4086 ± 0.03
	Adrenal Rt.	0.0086 ± 0.00	0.0092 ± 0.00	0.0089 ± 0.00	0.0079 ± ± 0.00	0.0076 ± 0.00	0.0078 ± 0.00
	Adrenal Lt.	0.0096 ± 0.00	0.0100 ± 0.00	0.0098 ± 0.00	0.0084 ± 0.00[a]	0.0081 ± 0.00	0.0097 ± 0.00[b]
	Testis Rt.	0.3934 ± 0.04	0.3955 ± 0.04	0.3944 ± 0.02	0.3762 ± 0.04	0.3684 ± 0.03	0.3799 ± 0.03
	Testis Lt.	0.3939 ± 0.04	0.3897 ± 0.05	0.3932 ± 0.02	0.3771 ± 0.05	0.3760 ± 0.04	0.3793 ± 0.03
	Epididymis Rt.	0.1261 ± 0.01	0.1235 ± 0.01	0.1202 ± 0.01	0.1183 ± 0.02	0.1229 ± 0.01	0.1202 ± 0.00
	Epididymis Lt.	0.1234 ± 0.02	0.1219 ± 0.01	0.1179 ± 0.01	0.1126 ± 0.02	0.1222 ± 0.01	0.1169 ± 0.01
	Prostate Gland	0.0904 ± 0.02	0.1083 ± 0.01	0.1002 ± 0.02	0.0986 ± 0.02	0.1074 ± 0.03	0.0857 ± 0.02
	Thymus	0.0570 ± 0.01	0.0573 ± 0.01	0.0610 ± 0.01	0.0576 ± 0.01	0.0595 ± 0.01	0.0610 ± 0.01
	Thyroid and parathyroid glands Rt.	0.0018 ± 0.00	0.0017 ± 0.00	0.0019 ± 0.00	0.0017 ± 0.00	0.0019 ± 0.00	0.0018 ± 0.00
	Thyroid and parathyroid glands Lt.	0.0019 ± 0.00	0.0018 ± 0.00	0.0022 ± 0.00	0.0019 ± 0.00	0.0017 ± 0.00	0.0023 ± 0.00[b]
	Pituitary gland	0.0024 ± 0.00	0.0021 ± 0.00	0.0023 ± 0.00	0.0020 ± 0.00	0.0023 ± 0.00	0.0023 ± 0.00
Female	Liver	2.6670 ± 0.19	2.6288 ± 0.16	2.6919 ± 0.24	2.6627 ± 0.13	2.5308 ± 0.08	2.6390 ± 0.22
	Kidney Rt.	0.2934 ± 0.02	0.3015 ± 0.02	0.2946 ± 0.02	0.2975 ± 0.02	0.2990 ± 0.01	0.2938 ± 0.01
	Kidney Lt.	0.2809 ± 0.02	0.2774 ± 0.01	0.2781 ± 0.02	0.2849 ± 0.02	0.2861 ± 0.01	0.2779 ± 0.01
	Heart	0.3659 ± 0.01	0.3602 ± 0.02	0.3684 ± 0.03	0.3572 ± 0.02	0.3591 ± 0.01	0.3549 ± 0.01
	Spleen	0.2300 ± 0.02	0.2373 ± 0.01	0.2420 ± 0.03	0.2413 ± 0.02	0.2427 ± 0.02	0.2404 ± 0.03
	Brain	0.7640 ± 0.05	0.7593 ± 0.05	0.7582 ± 0.05	0.7527 ± 0.04	0.7521 ± 0.04	0.7262 ± 0.03
	Adrenal Rt.	0.0194 ± 0.00	0.0198 ± 0.00	0.0183 ± 0.00	0.0186 ± 0.00	0.0158 ± 0.00	0.0174 ± 0.00
	Adrenal Lt.	0.0203 ± 0.00	0.0213 ± 0.00	0.0198 ± 0.00	0.0206 ± 0.00	0.0181 ± 0.00	0.0188 ± 0.00
	Ovaries and oviduct Rt.	0.0300 ± 0.00	0.0297 ± 0.00	0.0281 ± 0.01	0.0279 ± 0.00	0.0296 ± 0.00	0.0306 ± 0.00
	Ovaries and oviduct Lt.	0.0290 ± 0.00	0.0302 ± 0.00	0.0293 ± 0.00	0.0282 ± 0.00	0.0282 ± 0.00	0.0328 ± 0.00[b]
	Uterus	0.2377 ± 0.08	0.2813 ± 0.12	0.2202 ± 0.05	0.2453 ± 0.08	0.2328 ± 0.07	0.2502 ± 0.11
	Thymus	0.1012 ± 0.01	0.0855 ± 0.01	0.1074 ± 0.02	0.1086 ± 0.03	0.1003 ± 0.01	0.1050 ± 0.02
	Thyroid and parathyroid glands Rt.	0.0027 ± 0.00	0.0026 ± 0.00	0.0033 ± 0.00	0.0026 ± 0.00	0.0031 ± 0.00	0.0029 ± 0.00
	Thyroid and parathyroid glands Lt.	0.0029 ± 0.00	0.0028 ± 0.00	0.0032 ± 0.00	0.0028 ± 0.00	0.0029 ± 0.00	0.0031 ± 0.00
	Pituitary gland	0.0047 ± 0.00	0.0055 ± 0.00	0.0050 ± 0.00	0.0049 ± 0.00	0.0052 ± 0.00	0.0055 ± 0.00

Values are average ± standard deviation (n = 10, per group for male and female rats)
[a] The average is statistically significant difference at the *0.*05 levels of control group.
[b] The average is statistically significant difference at the *0.*05 levels of control-recovery group.

investigation is warranted to determine their relevance to the immunomodulatory effects of fingerroot extract. The estrus cycle of female rats was normal, and cytological evaluation showed that there were no abnormal cell types. Our microscopic examination revealed a hyaline cast and tubular basophilia in the kidney, but no evidence of ongoing hypertrophy, hyperplasia, or degeneration. In addition, we observed other lesions that are well known to occur in rats of the same age, and they were not dose dependent [39–42].

In this toxicity study, notable phenomena were observed with statistical significance, especially in medium and high-dose groups compared to the control. Although these occurrences were deemed clinical insignificant, they hold potential value for further study and the future product development of fingerroot extract formulation. Increasing the total exposure through higher doses and extended

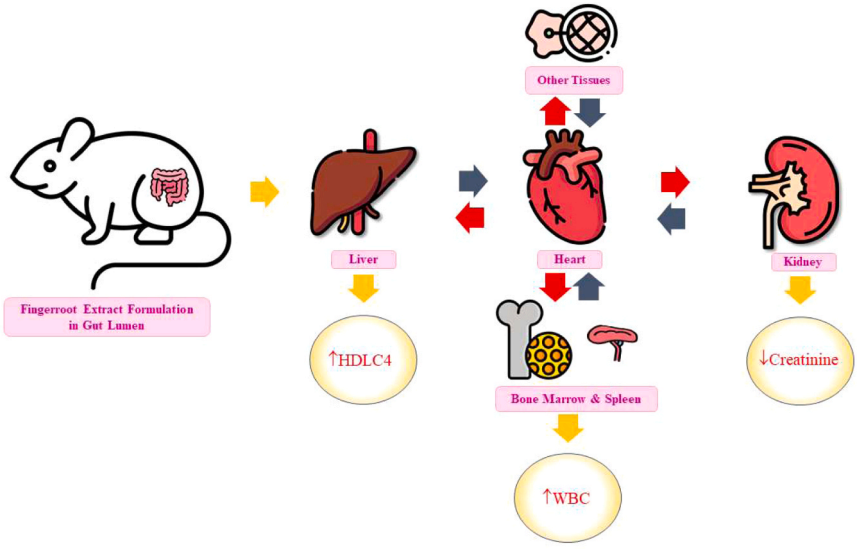

Fig. 4. A schematic diagram summarizing the modes of action determining the toxicity potential of fingerroot extract formulation in rats.

durations of the fingerroot extract formulation may elucidate these phenomena in terms of pharmacological or toxicological activities in exposed animals (Fig. 4). An increase in white blood cells was observed in both male and female rats in the medium and high-dose groups of the fingerroot extract formulation, compared to the control group. This could be attributed to the immunomodulatory effects of fingerroot extract in rodent models. Previous studies have reported decreases in inflammatory cytokines and modulation of bone metabolism following oral administration of fingerroot extract in rat and mouse models [43, 44]. Furthermore, a decrease in creatinine levels was observed in male rats receiving medium and high doses of fingerroot extract formulation. This phenomenon might be linked to the vasodilation effects of the fingerroot extract via the nitric oxide pathway and calcium channel blockade [45]. Given that creatinine values reflect the glomerular filtration rate of the kidney, increased blood supply could lead to lower creatinine levels. Statistically higher values of HDLC4 were observed across all groups of the fingerroot extract formulation in male rats. Numerous reports have indicated fingerroot extract's anti-adipogenic and anti-obesity properties over the past decade. Mechanisms such as activated protein kinase, regulation of lipid metabolism, reduced triglyceride, and increased HDLC4 were identified in rodent models with high-fat diets [46–48]. This toxicity study provides valuable insights and directs the future research of the fingerroot extract formulation. While no serious adverse events were detected in this experiment, further studies are urgently needed to minimize toxicity and maximize efficacy of fingerroot extract formulations as phytopharmaceutical products for the treatment of chronic inflammation.

5. Conclusion

Our results indicate no mortalities or toxicity in relation to dose response. While certain statistically significant changes were observed, these changes remained within the normal range observed in healthy rats. No serious adverse effects of the extract formulation were observed at doses up to 100 mg/kg throughout the 90-day study period under the experimental conditions. Consequently, the No Observed Adverse Effect Level (NOAEL) was established at 100 mg/kg/day.

Funding

This study was supported by the Program Management Unit Competitiveness (PMUC, Grant No. C17F640237), and Mahidol University (to Khemawoot P.).

CRediT authorship contribution statement

Khemawoot Phisit: Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Chang Leng Chee:** Writing – review & editing, Project administration, Conceptualization. **Wongwiwatthananukit Supakit:** Writing – review & editing, Project administration, Conceptualization. **Supannapan Kittitach:** Writing – review & editing, Methodology, Conceptualization. **Tangpanithandee Supawit:** Writing – review & editing, Writing – original draft, Project administration, Formal analysis, Data curation. **Techapichetvanich Pinnakarn:** Writing – review & editing, Writing – original draft, Project administration, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank all veterinarians at the National Laboratory Animal Center, Mahidol University, for conducting the animal experiments. We thank Associate Professor Pornpun Vivithanaporn for funding management of this project. This article has been proofread by PRS.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2024.01.013.

References

[1] L. Zhang, Q. Jiang, X. Wang, A. Jaisi, O.J. Olatunji, *Boesenbergia rotunda* displayed anti-inflammatory, antioxidant and anti-apoptotic efficacy in doxorubicin-induced cardiotoxicity in rats, Sci. Rep. 13 (2023) 11398, https://doi.org/10.1038/s41598-023-38560-5.

[2] N.M. Isa, S.I. Abdelwahab, S. Mohan, A.B. Abdul, M.A. Sukari, M.M.E. Taha, S. Syam, P. Narrima, S.C. Cheah, S. Ahmad, et al., *In vitro* anti-inflammatory, cytotoxic and antioxidant activities of boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (L.) (fingerroot), Braz. J. Med. Biol. Res. 45 (2012) 524–530, https://doi.org/10.1590/s0100-879×2012007500022.

[3] S. Mohan, Y.H. Hobani, E. Shaheen, A.S. Abou-Elhamd, A. abdelhaleem, H. A. Alhazmi, S.I. Abdelwahab, Ameliorative effect of boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (fingerroot) on oxidative stress and inflammation in ethanol-induced gastric ulcer in vivo, J. Ethnopharmacol. 261 (2020) 113104, https://doi.org/10.1016/j.jep.2020.113104.

[4] S.M. Salama, M. Bilgen, A.S. Al Rashdi, M.A. Abdulla, Efficacy of *Boesenbergia rotunda* treatment against thioacetamide-induced liver cirrhosis in a rat model, Evid. Based Complement Altern. Med. 2012 (2012) 137083, https://doi.org/10.1155/2012/137083.

[5] K. Shindo, M. Kato, A. Kinoshita, A. Kobayashi, Y. Koike, Analysis of antioxidant activities contained in the *Boesenbergia pandurata* Schult. rhizome, Biosci. Biotechnol. Biochem. 70 (2006) 2281–2284, https://doi.org/10.1271/bbb.60086.

[6] S.P. Voravuthikunchai, S. Phongpaichit, S. Subhadhirasakul, Evaluation of antibacterial activities of medicinal plants widely used among AIDS patients in Thailand, Pharm. Biol. 43 (2005) 701–706, https://doi.org/10.1080/13880200500385194.

[7] N.S. Zainin, K.Y. Lau, M. Zakaria, R. Son, A.F. Abdull Razis, Y. Rukayadi, Antibacterial activity of *Boesenbergia rotunda* (L.) Mansf. A. extract against *Escherichia coli*, Int. Food Res. J. 20 (2013) 3319–3323.

[8] S. Tewtrakul, S. Subhadhirasakul, C. Karalai, C. Ponglimanont, S. Cheenpracha, Anti-inflammatory effects of compounds from Kaempferia parviflora and Boesenbergia pandurata, Food Chem. 115 (2009) 534–538, https://doi.org/10.1016/j.foodchem.2008.12.057.

[9] H.T. San, H.E.E. Khine, B. Sritularak, E. Prompetchara, C. Chaotham, C.T. Che, K. Likhitwitayawuid, Pinostrobin: an adipogenic suppressor from fingerroot (boesenbergia rotunda) and its possible mechanisms, Foods 11 (2022), https://doi.org/10.3390/foods11193024.

[10] T. Eng-Chong, L. Yean-Kee, C. Chin-Fei, H. Choon-Han, W. Sher-Ming, C.T. Li-Ping, F. Gen-Teck, N. Khalid, N. Abd Rahman, S.A. Karsani, et al., *Boesenbergia rotunda*: from ethnomedicine to drug discovery, Evid. Based Complement Altern. Med 2012 (2012) 473637, https://doi.org/10.1155/2012/473637.

[11] J. Won, K. Noh, J.-K. Hwang, B.S. Shin, W. Kang, Pharmacokinetics of panduratin a following oral administration of a *Boesenbergia pandurata* extract to rats, J. Food Drug Anal. 29 (2021) 676.

[12] P. Kanjanasirirat, A. Suksatu, S. Manopwisedjaroen, B. Munyoo, P. Tuchinda, K. Jearawuttanakul, S. Seemakhan, S. Charoensutthivarakul, P. Wongtrakoongate, N. Rangkasenee, et al., High-content screening of Thai medicinal plants reveals *Boesenbergia rotunda* extract and its component Panduratin A as anti-SARS-CoV-2 agents, Sci. Rep. 10 (2020) 19963, https://doi.org/10.1038/s41598-020-77003-3.

[13] T. Kongratanapasert, S. Kongsomros, N. Arya, K. Sutummaporn, W. Wiriyarat, Y. Akkhawattanangkul, T. Boonyarattanasoonthorn, N. Asavapanumas, P. Kanjanasirirat, A. Suksatu, et al., Pharmacological activities of fingerroot extract and its phytoconstituents against SARS-CoV-2 infection in golden syrian hamsters, J. Exp. Pharm. 15 (2023) 13–26, https://doi.org/10.2147/jep.S382895.

[14] J.M. Bennett, G. Reeves, G.E. Billman, J.P. Sturmberg, Inflammation–Nature's way to efficiently respond to all types of challenges: implications for understanding and managing "the epidemic" of chronic diseases, Front. Med. 5 (2018), https://doi.org/10.3389/fmed.2018.00316.

[15] G. Schett, M.F. Neurath, Resolution of chronic inflammatory disease: universal and tissue-specific concepts, Nat. Commun. 9 (2018) 3261, https://doi.org/10.1038/s41467-018-05800-6.

[16] R.A. M, P.I. M, L. R, L. J, Inhibitory Activity of *Boesenbergia rotunda* (L.) Mansf. Rhizome towards the Expression of Akt and NF-KappaB p65 in Acetic Acid-Induced Wistar Rats, Evid. Based Complement Altern. Med. 2020 (2020) 6940313, https://doi.org/10.1155/2020/6940313.

[17] M. Syam, H.Y. Hasan, S. Emad, A.-E.A. Sayed, a; A. Aymen, A.H. Ibrahim, A.S, Ameliorative effect of Boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (Fingerroot) on oxidative stress and inflammation in ethanol-induced

gastric ulcer in vivo, J. Ethnopharmacol. 261 (2020) 113104, https://doi.org/10.1016/j.jep.2020.113104.
[18] J.-M. Yun, H. Kwon, J.-K. Hwang, In vitro anti-inflammatory activity of panduratin A isolated from *Kaempferia pandurata* in RAW264. 7 cells, Planta Med. 69 (2003) 1102–1108.
[19] Z. Shen, X. Bao, R. Wang, Clinical PET imaging of microglial activation: implications for microglial therapeutics in Alzheimer's disease, Front. Aging Neurosci. 10 (2018) 314.
[20] Y. Tang, W. Le, Differential roles of M1 and M2 microglia in neurodegenerative diseases, Mol. Neurobiol. 53 (2016) 1181–1194.
[21] S. Jamornwan, T. Chokpanuwat, K. Uppakara, S. Soodvilai, W. Saengsawang, Anti-inflammatory activity of panduratin A against LPS-induced microglial activation, Biomedicines 10 (2022) 2587.
[22] National Center for Biotechnology Information. PubChem Compound Summary for CID 73201, 5-Hydroxy-7-methoxy-2-phenylchroman-4-one. Available online: ⟨https://pubchem.ncbi.nlm.nih.gov/compound/5-Hydroxy-7-methoxy-2-phenylchroman-4-one⟩. (accessed on.
[23] A.M.G.K. Athapaththu, K.T. Lee, M.H.D. Kavinda, S. Lee, S. Kang, M.-H. Lee, C.-H. Kang, Y.H. Choi, G.-Y. Kim, Pinostrobin ameliorates lipopolysaccharide (LPS)-induced inflammation and endotoxemia by inhibiting LPS binding to the TLR4/MD2 complex, Biomed. Pharm. 156 (2022) 113874, https://doi.org/10.1016/j.biopha.2022.113874.
[24] N.K. Patel, K.K. Bhutani, Pinostrobin and cajanus lactone isolated from *Cajanus cajan* (L.) leaves inhibits TNF-α and IL-1β production: in vitro and in vivo experimentation, Phytomedicine 21 (2014) 946–953.
[25] M. Ekor, The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, Front. Pharmacol. 4 (2014), https://doi.org/10.3389/fphar.2013.00177.
[26] C.K. Robinson S, S. Hudson, S. Sparrow, D. Spencer-Briggs, A. Danks, R. Hill, D. Everett, B. Mulier, S. Old, Guidance on dose level selection for regulatory general toxicology studies for pharmaceuticals, NC3Rs/LASA, London, 2009.
[27] EUROPEAN MEDICINES AGENCY, S.M.H. ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals. 2009.
[28] P. Saraithong, S. Saenphet, K. Saenphet, Safety evaluation of ethanol extracts from *Bosenbergia rotunda* (L.) Mansf. in male rats, Trends Res. Sci. Technol. 2 (2010) 19–22.
[29] S. Charoensin, C. Punvittayagul, W. Pompimon, U. Meevatee, R. Wongpoomchai, Toxicological and clastogenic evaluation of pinocembrin and pinostrobin isolated from *Boesenbergia pandurata* in Wistar rats, Thai J. Toxicol. 25 (2010) 29–40.
[30] T. Boonyarattanasoonthorn, T. Kongratanapasert, A. Jiso, P. Techapichetvanich, N. Nuengchamnong, K. Supannapan, A. Kijtawornrat, P. Khemawoot, Absolute oral bioavailability and possible metabolic pathway of panduratin A from Boesenbergia rotunda extract in beagle dogs, Pharm. Biol. 61 (2023) 590–597, https://doi.org/10.1080/13880209.2023.2190777.
[31] O.E.C.D.. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents; 2018.
[32] J.F. Maspero, A.A. Cruz, C.F.P. Beltran, A. Ali Munive, F. Montero-Arias, R. Hernandez Pliego, H. Farouk, The use of systemic corticosteroids in asthma management in Latin American countries, World Allergy Organ J. 16 (2023) 100760, https://doi.org/10.1016/j.waojou.2023.100760.
[33] N.P. Patel, C.M. Bates, A. Patel, Developmental approaches to chronic pain: a narrative review, Cureus 15 (2023) e45238, https://doi.org/10.7759/cureus.45238.
[34] C. Chen, S.R. Haupert, L. Zimmermann, X. Shi, L.G. Fritsche, B. Mukherjee, Global prevalence of post-coronavirus disease 2019 (COVID-19) condition or long COVID: a meta-analysis and systematic review, J. Infect. Dis. 226 (2022) 1593–1607, https://doi.org/10.1093/infdis/jiac136.
[35] T. Meakleartmongkol, S. Tangpanithandee, N. Vanavivit, A. Jiso, P. Pongchaikul, S. Kirdlarp, P. Khemawoot, S. Nathisuwan, Potential drug-drug interactions of frequently prescribed medications in long COVID detected by two electronic databases, PLoS One 18 (2023) e0293866, https://doi.org/10.1371/journal.pone.0293866.
[36] M. Todić, S. Bakić, B. Begović, S. Krošnjar, I. Zulić, Food and water consumption in assessment of acute oral toxicity of HEPALIP FORTE in rats, Bosn. J. Basic Med. Sci. 3 (2003) 47–53, https://doi.org/10.17305/bjbms.2003.3493.
[37] Z. Linye, J. Qihong, W. Xiuming, J. Amit, O.O. Joshua, *Boesenbergia rotunda* displayed anti-inflammatory, antioxidant and anti-apoptotic efficacy in doxorubicin-induced cardiotoxicity in rats, Sci. Rep. 13 (2023) 11398, https://doi.org/10.1038/s41598-023-38560-5.
[38] S.M. Salama, I.A.A. Ibrahim, N. Shahzad, S. Al-Ghamdi, N. Ayoub, A.S. AlRashdi, M.A. Abdulla, N. Salehen, M. Bilgen, Hepatoprotectivity of Panduratin A against liver damage: In vivo demonstration with a rat model of cirrhosis induced by thioacetamide, APMIS 126 (2018) 710–721, https://doi.org/10.1111/apm.12878.
[39] D.G. Dunn, J.F.M. Baker, S.D. Sorden, Chapter 16 - Eye and Associated Glands, in: A.W., Ed Suttie (Ed.), In Boorman's Pathology of the Rat (Second Edition), Academic Press: Boston, 2018, pp. 251–278.
[40] K.S. Frazier, J.C. Seely, G.C. Hard, G. Betton, R. Burnett, S. Nakatsuji, A. Nishikawa, B. Durchfeld-Meyer, A. Bube, Proliferative and nonproliferative lesions of the rat and mouse urinary system, Toxicol. Pathol. 40 (2012) 14S–86S, https://doi.org/10.1177/0192623312438736.
[41] K. Shibuya, M. Tomohiro, S. Sasaki, S. Otake, Characteristics of structures and lesions of the eye in laboratory animals used in toxicity studies, J. Toxicol. Pathol. 28 (2015) 181–188, https://doi.org/10.1293/tox.2015-0037.
[42] B. Thoolen, R.R. Maronpot, T. Harada, A. Nyska, C. Rousseaux, T. Nolte, D. E. Malarkey, W. Kaufmann, K. Küttler, U. Deschl, et al., Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system, Toxicol. Pathol. 38 (2010) 5S–81S, https://doi.org/10.1177/0192623310386499.
[43] H. Kim, C. Kim, K.E. Kook, Choi, S. Yanti, W. Kang, J.K. Hwang, Inhibitory effects of standardized boesenbergia pandurata extract and its active compound panduratin A on Lipopolysaccharide-induced periodontal inflammation and alveolar bone loss in rats, J. Med. Food 21 (10) (2018) 961–970, https://doi.org/10.1089/jmf.2017.4155.
[44] M.S. Kim, H.B. Pyun, J.K. Hwang, Effects of orally administered fingerroot (*Boesenbergia pandurata*) extract on oxazolone-induced atopic dermatitis-like skin lesions in hairless mice, Food Sci. Biotechnol. 22 (Suppl 1) (2013) 257–264, https://doi.org/10.1007/s10068-013-0075-z.
[45] D. Adhikari, D.S. Gong, S.H. Oh, E.H. Sung, S.O. Lee, D.-W. Kim, M.-H. Oak, H. J. Kim, Vasorelaxant effect of *Boesenbergia rotunda* and its active ingredients on an isolated coronary artery, Plants 9 (2020) 1688, https://doi.org/10.3390/plants9121688.
[46] S. Fattepur, K. Nilugal, R. Rajendran, F. Asmani, E. Yusuf, Anti-hyperlipidemic activity of methanolic extract of Boesenbergia pandurata (finger root) in experimental induced hypercholestrolemic sprague dawley rats, Asian J. Pharm. Clin. Res 11 (2018) 8, https://doi.org/10.22159/ajpcr.2018.v11s3.29962.
[47] D.Y. Kim, M.S. Kim, B.K. Sa, M.B. Kim, J.K. Hwang, Boesenbergia pandurata attenuates diet-induced obesity by activating AMP-activated protein kinase and regulating lipid metabolism, Int. J. Mol. Sci. 13 (1) (2012) 994–1005, https://doi.org/10.3390/ijms13010994.
[48] K.S. Myoung, Y.T. Ahn, M.H. Lee, D.Y. Park, Y.M. Ahn, C.S. Huh, Fingerroot (Boesenbergia pandurata) extract inhibits the accumulation of visceral fat in C57BL/6J mice, J. Korean Soc. Food Sci. Nutr. 42 (2013) 26–32, https://doi.org/10.3746/jkfn.2013.42.1.026.