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Research article

Acute oral toxicity study of seed, peel and pulp extracts from Ha Chau Burmese-grape (*Baccaurea ramiflora* Lour.) fruit using mice

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Abstract

Importance of the work: The first evidence of safety for human consumption was provided for Ha Chau (*Baccaurea ramiflora* Lour.) fruit extracts, addressing a critical gap in toxicological data.

Objectives: To determine the acute oral toxicity profile of Ha Chau fruit peel, pulp and seed extracts in mice.

Materials and Methods: Swiss albino mice received oral doses of ethanolic peel extract, ethanolic seed extract or pulp juice (5–5,000 mg/kg) using a fixed-dose procedure. Clinical signs, biochemical and hematological indices, organ weights and histopathology were evaluated for 14 d following the respective treatments.

Results: All the treated groups had 100% survival with a dose above 5,000 mg/kg required to kill one-half of the tested members. The tested biochemical and hematological parameters remained within normal ranges. Organ weights (liver, kidney, spleen), morphological characteristics and histological findings were comparable between the treatment and control groups, indicating the absence of extract-induced alterations.

Main finding: The Ha Chau fruit peel, pulp and seed extracts exhibited no acute toxicity at high doses, providing novel toxicological evidence supporting their safe use in functional food and pharmaceutical applications.

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Introduction

Ha Chau (*Baccaurea ramiflora* Lour.) is found in Burma, Cambodia, Laos, Vietnam, India, Bangladesh and Malaysia (Niu et al., 2022). The juice from its fruit has high concentrations of phenolic and flavonoid chemicals, with possible natural antioxidant activity (Uddin et al., 2018; Xuan and Ha, 2023). The peel of the mature fruit has a high content of pectin and has been used to make jellies and jams (Hossain et al., 2017). According to Hasan et al. (2009), the medicinal value of the fruit had antiviral properties and could be used for skin disease treatment. In Vietnam, the Ha Chau variety has been selected and propagated from the local varieties of *B. ramiflora* in Phong Dien District, Can Tho City (Xuan and Nguyen, 2022). In 2006, Ha Chau fruit (HCF) was recognized with a trademark by the Intellectual Property Office of Vietnam (Xuan and Nguyen, 2022). The main harvest season of HCF occurs during the 7th–8th lunar months (approximately August–September in the Gregorian calendar), with two minor harvest periods in the 6th and 10th lunar months (approximately July and October) (Xuan and Nguyen, 2022). HCF is popular as fresh pulp with juice, while the seeds are swallowed after the peel has been removed; alternatively, the whole fruit is used in the preparation of dishes (Hossain et al., 2017).

In recent years, there has been expanded recognition and subsequent increased consumption of fruits and vegetables as sources of natural nutrients and non-nutritive food ingredients, as well as for disease prevention (Slavin and Lloyd, 2012). While, the use of herbal medicines and phytonutrients or nutraceuticals is expanding rapidly all over the world, toxicological assessment is necessary as a part of the developmental process for the use of plant extracts in the food, cosmetic and pharmaceutical industries and any new drug must be clinically tested and evaluated for toxicity before clinical use (Vaghasiya et al., 2011; Gohil et al., 2022). Toxicological evidence has confirmed that any substance is potentially toxic unless the correct dose, absorbed dose and duration of exposure are determined (United States Food and Drug Administration, 2000). To date, the limited published data on the dose and health effects of consuming HCF includes a lack of information regarding the mechanism of action, side effects and contraindications for the safe use of HCF.

B. ramiflora is a nutritious fruit rich in phenolics, flavonoids and saponins, with antioxidant and medicinal properties, suggesting its potential use in health products (Hossain et al., 2017). However, safety data remain scarce; while the cytotoxic effects of pulp and seed extracts have not been reported, there was no toxicity in leaf and bark extracts up to 5,000 mg/kg (Saha et al., 2017; Nesa et al., 2018; Choudhury et al., 2021). Furthermore, based on a 90 day study, there were no adverse effects at 400 mg/kg/day in mice (Xuan and Nguyen, 2022).

Consequently, the current study used a mouse model to determine the acute oral toxicity of peel, pulp and seed extracts from HCF according to the Organization for Economic Cooperation and Development (OECD) Test No. 420 Guidelines (Organization for Economic Cooperation and Development, 2002) and Kandimalla et al. (2016). This information should serve as an important foundation for evaluating the preventative potential of an HCF-rich diet.

Materials and Methods

Plant collection

Bunches of fresh HCF were harvested three times at the ripe stage in August, in Phong Dien District, Can Tho City, Vietnam. These bunches were encased in plastic bags with Ha Chau leaves and transferred swiftly to the laboratory. After removing any diseased, decayed and defective fruits, the remaining individual fruits were rinsed in tap water to eliminate foreign matter and dust and then air-dried thoroughly before the experiments. Then, the ripe HCF sample was separated into the peel, juice and seed.

Extract preparation

All the extract procedures were conducted according to Bahrin et al. (2018) and the Ministry of Health (2017), with modifications. HCF peel samples were cut into small pieces and dried at 50 ± 2°C for 8 hr in a hot-air oven (Memmert KS19; Germany) to a final 8−10% moisture content. The HCF seed samples were dried similarly to the peel sample but for 12 h. After drying, the separate parts were ground into powder, 200 g of each part were soaked in 4 L of 96% ethanol for 24 hr at room temperature and then passed through Whatman No. 1 filter paper. The filtrate from each extract was evaporated at 58 ± 2°C using a rotary evaporator (Ika, model RV10 digital V; Germany). HCF juice was obtained from squeezing the pulp and passing through Whatman No. 1 filter paper and evaporating at 58 ± 2°C to obtain the pulp extract with approximately 10% moisture content. The peel, pulp and seed extracts were evaluated separately for acute oral toxicity in mice.

Before oral administration, each type of extract was individually dissolved in 1% (volume per volume, v/v) dimethyl sulfoxide (DMSO), which was used as a safe vehicle according to OECD Test No. 420 Guidelines (Organization for Economic Cooperation and Development, 2002). The three extracts were tested separately with no combinations considered. The solutions were adjusted to provide the target dose levels (5 mg/kg body weight, 50 mg/kg body weight, 300 mg/kg body weight, 2,000 mg/kg body weight and 5,000 mg/kg body weight). The dose levels were determined in accordance with the OECD Test No. 420 Guidelines for Fixed-Dose Procedure (Organization for Economic Cooperation and Development, 2002), which provides standard dose levels to identify potential toxicity without prior estimation of the dose required to kill one-half of the members of a tested population (LD_{50}), ensuring a broad safety assessment range beyond normal dietary exposure. In addition, relevant information was considered from other studies regarding dose selection to ensure the safe use of *B. ramiflora* (Saha et al., 2017; Nesa et al., 2018; Choudhury et al., 2021; Xuan and Nguyen, 2022).

Animal ethics approval

The study was carried out with the permission of the Council for Science and Education, Can Tho University, Vietnam (No: DHC2021–01/KNN).

Acute oral toxicity assay

Acute oral toxicity was conducted according to the OECD Test No. 420 Guidelines (Organization for Economic Cooperation and Development, 2002) and Kandimalla et al. (2016) with modifications. Distilled water was used as the control, as has been applied in other acute toxicity studies (Gatsing et al., 2005; Sureshkumar et al., 2018). Notably, Xuan and Nguyen (2002) gave mice distilled water once a day for 90 d and reported no abnormal signs of digestive disorders. Healthy, male Swiss albino mice (18−22 g, aged 5-6 wk) obtained from the Institute of Vaccines and Medical Biologicals (IVAC, Vietnam) were used for the acute oral toxicity test. Before the experiment was conducted, the mice were housed in an experimental facility and given water and pellet feed, according to the IVAC guidelines (Institute of Vaccines and Medical Biologicals, 2018) for 1 wk. Each mouse was weighed and then allocated randomly into the control, DMSO and one of the five groups for each extract type (peel, pulp and seed treatments). The animal was once orally provided with distilled water (the control group), DMSO 1% (v/v) concentration (DMSO group), or 5 mg/kg body weight, 50 mg/kg body weight, 300 mg/kg body weight, 2,000 mg/kg body weight or 5,000 mg/kg body weight of peel, pulp and seed extract (treatment groups). In total, 85 mice were used, consisting of 5 mice for the control group, 5 mice for the DMSO group and 75 mice for the treatment groups (5 mice per dose level for each extract type of peel, pulp and seed). The initial dose level of the extracts was selected to observe individual toxicity signs at 30 min, 4.5 hr, 9 hr, 18 hr, 36 hr and 72 hr. If the animals displayed no signs of toxicity, the dose was increased until the toxic dose was determined. After 14 d of dosage, the body and organs (liver, kidney, spleen) weights of all animals were recorded and compared to the control group. The histopathological examination was conducted after the mice liver, kidney and spleen had been soaked in 10% formalin. In particular, the group of mice fed the extract at the highest dose once via oral gavage had blood samples collected using heart puncture for biochemical and hematological analysis.

Detection of toxicity signs

The signs of toxicity in each mouse were evaluated based on changes in the skin and fur, eyes and mucous membranes, salivation, respiratory, urination, diarrhea, activity and behavior, sleep and coma, tremors and convulsions, itchiness, lethargy and mortality. These signs were observed at least once during the first 30 min, periodically during the first 24 hr, (with special attention given during the first 4 hr) and daily thereafter, for a total of 14 d (Organization for Economic Cooperation and Development, 2002). On the 15th day, each mouse was weighed before being euthanized to determine organ (liver, kidney, spleen) weights to detect any probable harmful effects at the macroscopic level. The percentage body weight change for each mouse was calculated using Equation 1:

$$\text{Percentage body weight change} = (\text{Body weight on sacrifice day} - \text{Body weight on day of dosing}) / (\text{Body weight on day of dosing}) \times 100 \quad (1)$$

where all weights are measured in grams.

The percentage of organ weight to body change was calculated using Equation (2):

$$\text{Percentage organ weight to body weight} = \text{Organ weight} / \text{Body weight on sacrifice day} \quad (2)$$

where all weights are measured in grams.

In addition, the LD_{50} dose was recorded and the number of deaths at each dose.

Examination of exterior morphological characteristics of organs

The skin and abdominal wall of each mouse carcass were dissected to expose the abdominal viscera. The liver, kidney and spleen in the abdomen were identified and examined. Changes (if any) in color, size, quantity, location, bleeding or other abnormalities of these organs were recorded carefully (Organization for Economic Cooperation and Development, 2002; Kandimalla et al., 2016).

Biochemical analysis

The evaluated biochemical parameters were: aspartate aminotransferase AST and alanine aminotransferase ALT, both measured in Units per liter; creatinine measured in micro moles per liter; and urea, cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C), all measured in milligrams per deciliter. The semi-automated biochemistry analyzer (ERBA Chem–7; Germany) was used in this process.

Hematological analysis

The measured hematological parameters were: white blood cells (WBC), measured in thousands per cubic millimeter ($10^3/mm^3$); neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BASO) and red blood cells (RBC), all measured as percentages; hemoglobin (HGB), measured in grams per deciliter; hematocrit (HCT), measured in liters per liter; mean corpuscular hemoglobin (MCH) measured in picograms; mean corpuscular hemoglobin concentration (MCHC), measured in grams per liter; and platelet count (PLT), measured in thousands per cubic millimeter. The semi-automated biochemistry analyzer (ERBA Chem–7; Germany) was used in this process.

Histopathological study

The liver and kidneys of each mouse were cleaned with normal saline and then promptly fixed with 4% formalin. To create tissue slices, these organs were dehydrated in increasing concentrations of ethanol (50–99.5%), cleaned in xylene, fixed in paraffin and sectioned at 3 µm using a rotary microtome (Leica Microsystems, Germany). After being treated in an alcohol-xylene series, the tissue sections were stained in hematoxylin and eosin Y dye before being examined under a microscope (cx21fs1; Olympus Corp; Japan), according to Saleem et al. (2017).

Statistical analysis

Statistical analyses were performed using a one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test, using Minitab 16 software (Minitab Inc., State College, PA, USA). Differences were considered statistically significant at $p < 0.05$. Results are presented as mean ± SD.

Results and Discussion

The results of acute oral toxicity are required to demonstrate that the test is safe regarding human health and aid in determining an acceptable starting dose (Organization for Economic Cooperation and Development, 2002).

Observation of toxicity signs

Mice given fixed dosages of HCF seed, peel and pulp extracts at 5 mg/kg, 50 mg/kg, 300 mg/kg, 2,000 mg/kg and 5,000 mg/kg survived and showed no signs of discomfort for up to 14 hr after dosing (Table 1). All mice consumed food and water normally throughout the trial period, indicating that the three types of extracts at different doses did not influence the digestive system and did not disturb the carbohydrate, protein or fat metabolism in the bodies of mice in each of the treatment groups (Casarett et al., 2001; Chokshi, 2007). During the 14 d observation period, the skin and fur, eyes and mucous membranes, salivation, respiration, urine, diarrhea, activity and behavior, sleep and coma indications of both groups of treated mice were normal and free of tremors and convulsions, itching and lethargy (Table 1). Therefore, oral administration of the seed, peel and pulp extracts from HCF had low toxicity to mice and the LD_{50} was determined to be greater than 5,000 mg/kg. According to the Organization for Economic Cooperation and Development (2002) and Kennedy et al. (1986), substances were regarded as being safe or practically nontoxic with LD_{50} values higher than 5,000 mg/kg by the oral route.

Table 1 Behavioral patterns of mice in control and treated groups during 14 d.

Treatment group	Parameter of behavior											
	S–F	E–M	S	R	U	D	A–B	S–C	T–C	I	L	Dead
Control	N	N	N	N	N	N	N	N	NF	NF	NF	NF
DMSO 1%	N	N	N	N	N	N	N	N	NF	NF	NF	NF
Single dose of seed extract (SE)												
5 mg/kg SE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
50 mg/kg SE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
300 mg/kg SE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
2,000 mg/kg SE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
5,000 mg/kg SE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
Single dose of peel extract (PE)												
5 mg/kg PE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
50 mg/kg PE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
300 mg/kg PE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
2,000 mg/kg PE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
5,000 mg/kg PE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
Single dose of pulp extract (PuE)												
5 mg/kg PuE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
50 mg/kg PuE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
300 mg/kg PuE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
2,000 mg/kg PuE	N	N	N	N	N	N	N	N	NF	NF	NF	NF
5,000 mg/kg PuE	N	N	N	N	N	N	N	N	NF	NF	NF	NF

DMSO = dimethyl sulfoxide; N = normal, NF = not found; S–F: skin and fur; E–M: eyes and mucous membranes; S: salivation; R: respiratory; U: urination; D: diarrhea; A–B: activity and behavior; S–C: sleep and coma; T–C: tremors and convulsions; I: itchiness; L: lethargy.

As shown in Table 2, the body weight growth varied from 6.28 ± 0.83% to 9.19 ± 1.04%, indicating an increase in mice body weight following the various dosages. The animals given the highest dose of HCF seed extract (5,000 mg/kg) achieved the highest significant body weight gain (9.19 ± 1.04%) compared to the control group. The rapid weight gains in the seed extract-fed mice compared with those given other extracts could have been due to the oil content (25%) in the seed of *B. ramiflora* (Gogoi, 2017). This was supported by the concentration of triglycerides (321.77 ± 36.61 mg/dL) (Table 3) in the serum of the treated group (seed extract) at a dose of 5,000 mg/kg that was 1.62 times and 2.41 times greater than the groups using the peel and pulp extracts, respectively, at the same dose. The peel extract had the lowest percentage body weight gain in the test animals (6.28 ± 0.83%). In addition, the peel extract encouraged not only a reduction in triglycerides but also increased the level of HDL-cholesterol in the mouse serum tests, leading to regulating of the blood lipid indexes and avoiding quick weight gain.

According to Vaghasiya et al. (2011), the relative organ weight, an indicator of a physiological and pathological condition in humans and animals, was a useful measure for assessment of organ damage. Often, impaired organs have abnormal tumidity or atrophy (Wang et al., 2007). Dybing et al. (2002) reported that the liver, kidney and spleen were the first organs influenced by toxic substances through metabolic reactions. In the present study, the relative organ weights of the mice in the treatment group were similar to those of the control group. The percentages of organ weight to body weights were in the ranges 8.45 ± 0.75–9.75 ± 1.40% (liver weight/body weight), 1.59 ± 0.29–2.13 ± 0.29% (kidney weight/body weight) and 0.43 ± 0.01–0.48 ± 0.01% (spleen weight/body weight) in the mice with any dose of the extracts.

HCF has a sweet taste that overwhelms less intense the sour taste, so it is necessary to check the blood glucose level after consuming the fruit. Table 2 shows that the three extracts at 5 mg/kg, 50 mg/kg, 300 mg/kg, 2,000 mg/kg and 5,000 mg/kg had no significant effect on the blood glucose level of the mice from the control and treatment groups after 14 d, with the figures in the range 114.48 ± 4.33-126.72±4.86 mg/dL.

Table 2 Effects on relative organ-to-body weight and blood glucose level in mice for control and treated groups after 14 d from initial dosing.

Treatment group	Parameter (%)				
	Body weight gain	Relative organ weight (%)			Glucose (mg/dL)
		Liver weight (per body weight)	Kidney weight (per body weight)	Spleen weight (per body weight)	
Control	7.42±0.51^{bc}	9.13±1.80^{ab}	1.71±0.21^{bc}	0.45±0.03^{cde}	117.72±2.41^{ab}
DMSO 1%	6.91±0.75^{cd}	9.43±0.62^{ab}	1.69±0.22^{bc}	0.44±0.02^{def}	126.72±4.86^{a}
Single dose of seed extract (SE)					
5 mg/kg SE	7.53±0.91^{bc}	8.45±0.75^{b}	1.89±0.20^{abc}	0.43±0.01^{f}	119.52±4.33^{ab}
50 mg/kg SE	7.89±0.71^{b}	9.39±0.66^{ab}	1.89±0.36^{abc}	0.45±0.01^{cde}	118.44±3.90^{ab}
300 mg/kg SE	7.75±0.73^{bc}	9.17±0.40^{ab}	1.62±0.27^{c}	0.45±0.01^{cde}	120.96±4.66^{ab}
2,000 mg/kg SE	8.16±0.46^{b}	9.24±0.36^{ab}	1.69±0.22^{bc}	0.44±0.01^{def}	123.12±4.86^{ab}
5,000 mg/kg SE	9.19±1.04^{a}	9.75±1.40^{a}	1.66±0.22^{bc}	0.46±0.01^{ad}	114.48±4.33^{b}
Single dose of peel extract (PE)					
5 mg/kg PE	7.82±0.86^{bc}	8.90±0.67^{ab}	2.13±0.29^{a}	0.48±0.01^{a}	123.12±4.86^{ab}
50 mg/kg PE	7.52±0.79^{bc}	9.26±0.43^{ab}	1.89±0.31^{abc}	0.47±0.01^{ab}	118.44±4.99^{ab}
300 mg/kg PE	7.42±0.45^{bc}	9.46±0.40^{ab}	1.92±0.33^{abc}	0.46±0.02^{bcd}	120.96±3.69^{ab}
2,000 mg/kg PE	7.42±0.86^{bc}	8.72±0.53^{ab}	1.64±0.27^{bc}	0.45±0.01^{bcd}	118.80±4.93^{ab}
5,000 mg/kg PE	6.28±0.83^{b}	8.51±1.34^{b}	1.59±0.29^{c}	0.47±0.03^{ad}	123.48±6.92^{b}
Single dose of pulp extract (PuE)					
5 mg/kg PuE	7.70±1.02^{bc}	8.78±0.70^{ab}	2.08±0.36^{a}	0.45±0.01^{bcd}	118.44±4.48^{ab}
50 mg/kg PuE	7.93±0.45^{bc}	9.48±0.63^{ab}	1.79±0.33^{abc}	0.45±0.01^{cf}	120.24±4.66^{ab}
300 mg/kg PuE	7.28±0.84^{bcd}	9.46±0.78^{ab}	1.98±0.23^{ab}	0.43±0.01^{ef}	120.96±7.13^{ab}
2,000 mg/kg PuE	7.19±0.67^{bcd}	9.23±0.27^{ab}	1.68±0.24^{bc}	0.47±0.01^{abc}	119.16±3.90^{ab}
5,000 mg/kg PuE	7.54±1.35^{bc}	8.80±0.85^{ab}	1.65±0.25^{bc}	0.45±0.02^{bcd}	119.52±3.27^{ab}

DMSO = dimethyl sulfoxide.

Values (mean ± SD) with different lowercase superscripts within same column are significantly ($p < 0.05$) different. Each treatment group contained 5 mice.

Table 3 Effects of extract (at dose level of 5,000 mg/kg) on liver and renal function tests, lipid profile and complete blood count in mice

Parameter	Control	DMSO 1%	HCF extract at dose level of 5,000 mg/kg		
			Seed	Peel	Pulp
Liver function test					
AST (U/L)	109.60±12.46^{a}	138.00±14.16^{a}	143.40±15.11^{a}	135.80±32.76^{a}	135.40±34.66^{a}
ALT (U/L)	92.00±3.24^{a}	107.20±5.67^{a}	99.40±18.66^{a}	111.80±6.72^{a}	120.00±30.32^{a}
Renal function test					
Urea (mg/dL)	8.23±1.37^{a}	8.81±0.59^{a}	10.53±1.85^{a}	10.01±1.14^{a}	5.74±1.15^{b}
Createnine (µmol/L)	45.80±4.76^{a}	48.00±3.67^{a}	47.80±1.30^{a}	47.40±5.23^{a}	47.00±5.66^{a}
Lipid profile					
Cholesterol (mg/dL)	120.54±4.05^{a}	122.93±13.21^{a}	114.83±5.88^{a}	119.38±8.44^{a}	119.31±12.58^{a}
Triglycerides (mg/dL)	232.21±27.02^{b}	233.45±44.51^{b}	321.77±36.61^{a}	198.41±15.51^{b}	133.45±9.29^{c}
HDL-C (mg/dL)	6.73±1.30^{c}	4.74±0.85^{c}	6.80±2.26^{c}	16.91±1.35^{b}	29.81±6.62^{a}
LDL-C (mg/dL)	25.71±5.01^{b}	24.71±7.44^{bc}	13.44±2.91^{c}	29.34±7.78^{ab}	39.00±5.72^{a}
Complete blood count (CBC)					
WBC (10^3/mm^3)	4.92±0.76^{a}	5.54±0.69^{a}	4.39±0.92^{ab}	5.21±0.54^{a}	3.35±0.33^{b}
NEU (%)	12.98±2.14^{a}	13.38±1.12^{a}	7.84±0.87^{b}	11.50±1.90^{a}	6.50±0.80^{b}
LYM (%)	81.94±3.47^{a}	82.02±3.89^{a}	84.78±4.15^{a}	84.10±2.40^{a}	86.28±3.03^{a}
MON (%)	2.90±0.70^{b}	5.10±1.70^{a}	2.42±0.71^{b}	3.38±1.07^{ab}	1.48±0.15^{b}
EOS (%)	0.10±0.00^{b}	0.10±0.00^{b}	0.22±0.04^{a}	0.20±0.10^{a}	0.10±0.00^{b}
BASO (%)	1.92±0.30^{b}	2.46±0.25^{a}	2.44±0.40^{ab}	2.48±0.24^{a}	2.16±0.11^{ab}
RBC (%)	10.10±0.76^{a}	10.19±0.85^{a}	9.37±0.54^{a}	9.21±0.50^{a}	9.94± 0.99^{a}
HGB (g/dL)	134.00±4.06^{a}	139.80±5.89^{a}	137.40± 5.22^{a}	129.40±9.24^{a}	136.60±8.76^{a}
HCT (L/L)	0.52±0.03^{a}	0.53±0.06^{a}	0.50±0.03^{a}	0.48±0.04^{a}	0.52±0.06^{a}
MCH (pg)	13.58±1.10^{a}	13.96± 1.24^{a}	14.70 ±0.51^{a}	14.06±0.59^{a}	13.86±0.82^{a}
MCHC (g/L)	256.80±16.57^{a}	257.40± 22.07^{a}	272.80±9.07^{a}	271.60±8.93^{a}	267.40±18.96^{a}
PLT (10^3/mm^3)	1123.60±84.20^{a}	791.60±56.00^{b}	1108.60±142.20^{a}	1238.00±137.70^{a}	1167.80±57.70^{a}

HCF = Ha Chau fruit; AST = aspartate aminotransferase; ALT = alanine aminotransferase; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; WBC = white blood cell count; NEU = neutrophils; MON = monocytes; EOS = eosinophils; BASO = basophils; RBC = red blood cell count; HGB = hemoglobin concentration; HCT = hematocrit; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet count.

Values (mean ± SD) with different lowercase superscripts within same row are significantly ($p < 0.05$) different.

Evaluation of external morphological features of organs

Representative images of the liver, kidney and spleen samples from each mouse group are shown in Fig. 1. There were no external morphological changes in any of these organs in the mice from the groups fed seed, peel or pulp extracts with a dose of 5,000 mg/kg body weight compared to the control group. The liver surfaces a normal brown color, with a surface and clearly divided lobes. The kidneys had a typical size, with a smooth surface and no aberrant characteristics. The spleen, which plays a crucial role in blood filtration, had a typical average size and smooth surface. Altogether, the HCF extracts had no apparent effect on the external morphological features of these mice organs based on acute oral toxicity testing.

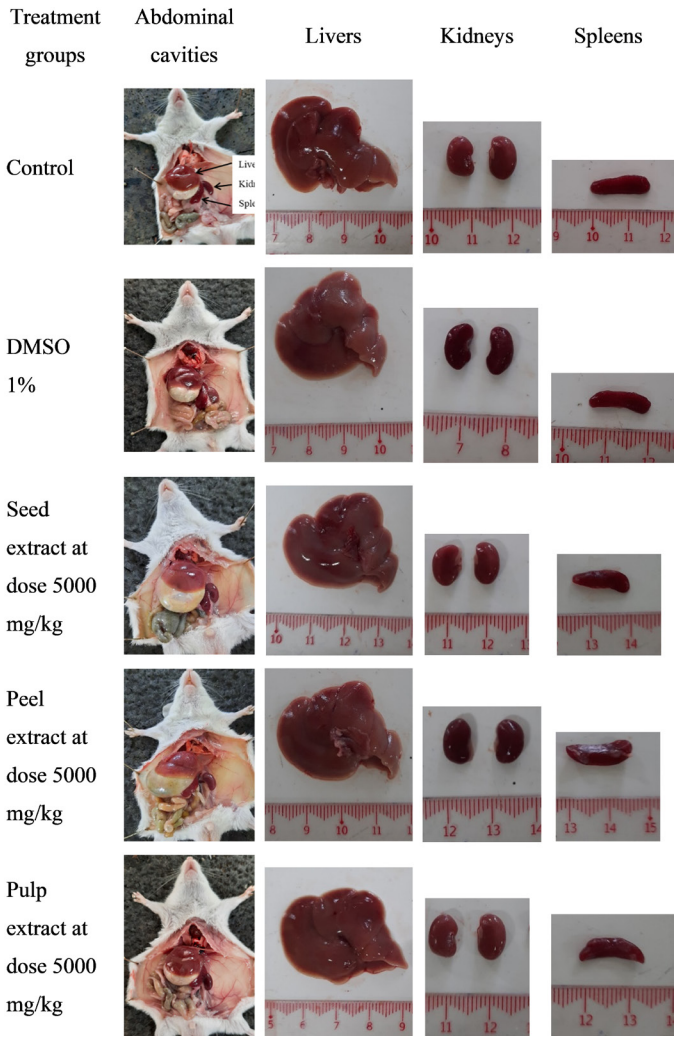

Fig. 1 Liver, kidney and spleen samples isolated from different mouse treatment groups following acute toxicity test at dose of 5,000 mg/kg body weight, where DMSO = dimethyl sulfoxide and scale unit is centimeters.

Biochemical analysis

The liver and kidneys play important roles in food metabolism and waste excretion (Sureshkumar et al., 2018). Therefore, in evaluating the toxicity of the extracts, the status of these organs needed to be determined by biochemical analysis (Sureshkumar et al., 2018). For liver function, serum levels of the two enzymes AST and ALT are commonly used as clinical, biochemical markers associated with liver injury (Bouhrim et al., 2018). When the liver is damaged, it allows different enzymes in the cytosol to be released into the blood stream and the transaminase activity of the AST and ALT enzymes increases (Gatsing et al., 2005). In Table 3, the AST and ALT levels in the seed treatment (143.40 ± 15.11 U/L and 99.40 ± 18.66 U/L, respectively), in the peel treatment (135,80 ± 32.76 U/L and 111.80 ± 6.72 U/L) and in the pulp treatment (135.40 ± 34.66 U/L and 120.00 ± 30.32 U/L) in the serum of the groups of mice using extracts at dose 5,000 mg/kg were not significantly different to the levels of these enzymes (109.60 ± 12.46 U/L and 92.00 ± 3.24 U/L) in the serum of the control group of mice. This indicated that the extracts from the seed, peel and pulp did not affect the liver function because the levels of these two enzymes were within the normal ranges for this species (Vernau, 2005). The urea and creatinine blood tests were used to assess kidney function. As shown in Table 3, there were no changes in the creatinine concentration between the treatment and control groups. The same pattern was evident in the urea content of both mice using seed and peel extracts compared to the mice in the control group, with figures in the range 8.23 ± 1.37–10.53 ± 1.85 mg/dL. More specifically, the decrease in the urea content (5.74 ± 1.15 mg/dL) of the group of mice fed the pulp extract at a dose of 5,000 mg/kg was significantly different from that of the control group. This was a positive sign of the benefits of the HCF pulp extract for kidney function.The main types of blood lipids, such as HDL-C, LDL-C, cholesterol and triglycerides in the experimental mice groups, are shown in Table 3. The cholesterol contents were in the range 114.83 ± 5.88-119.38±8.44 mg/dL in the mice fed the extracts from the seed, pulp or peel with a dose of 5,000 mg/kg, which were not significantly different from those of the control group (120.54 ± 4.05 mg/dL). The triglyceride levels in the group of mice using seed extract increased to 321.77 ± 36.61 mg/dL, which was 1.39 times greater than the control group (232.21 ± 27.02 mg/dL). The opposite pattern was evident in the triglyceride contents between the treatment and control groups. In detail, this content decreased to 198.41 ± 15.51 mg/dL with the peel extract and to 133.45 ± 9.29 mg/dL for the pulp extract,

indicating that the peel and pulp extracts could be useful in reducing blood triglycerides and as a potential agent for the regulation of blood lipids. The blood HDL-C contents (Table 3) rose to 16.91 ± 1.35 mg/dL and 29.812 ± 6.62 mg/dL for the groups fed the peel and pulp extracts, respectively, representing increases of about 2.51 and 4.43 times, respectively, compared to the control group (6.73 ± 1.30 mg/dL). There was no significant difference in the blood HDL-C levels between the group fed the seed extract compared to the control group. Furthermore, the blood LDL-C contents were in the range 13.44 ± 2.91–39.00 ± 5.72 mg/dL for all groups of mice. In particular, including the HCF seed extract in the diet reduced the LDL-C content by 1.91 times compared to the control group. In summary, the HCF reduced the level of LDL ("bad cholesterol") and increased the level of HDL ("good cholesterol) in the blood.

Hematological analysis

Hematological parameters are considered sensitive and reliable indicators to detect the intrusion of toxic substances (Rahman et al., 2001). Table 3 shows there were similar concentrations of WBC, NEU, LYM, MON, RBC, HGB, HCT, MCH, MCHC and PLT in the common blood tests among the groups of mice fed HCF extracts at a dose of 5,000 mg/kg and the control. This showed that the HCF extracts could be considered generally as safe. Leukocytes or WBC are an important part of the body's immune system, forming the first line of defense to protect the body from infectious agents, tissue damage or inflammatory processes. Based on the results of the present study, there was a significant decrease in WBC ($3.35 \pm 0.33 \times 10^3/mm^3$) and increase in LYM (86.28 ± 3.03%) in the mice fed the pulp extract, showing the immune-stimulating properties of the pulp extract. Govind et al. (2012) noted that the presence of saponins, flavonoids and alkaloids in plants enhanced immune function that stimulated lymphocyte proliferation. As a whole, the results of the present study demonstrated that HCF seed, peel and pulp extracts could be considered non-toxic.

Histopathological study

The liver and kidneys are two important organs that play a major metabolic role in stabilizing the state of the body to excrete metabolic wastes (Nguenang et al., 2020). The kidneys of adult mice receive 9–22% of the cardiac output (Stott et al., 1983); however, based on the results of the present study (Table 2) the kidney weight per body weight was just 1.59±0.29–2.13±0.29, so they are prone to metabolic disorders and can easily accumulate toxic substances. When the kidneys are exposed to poisonous agents, the renal tubules may be damaged (Bayomy et al., 2017). In the present study, the liver and kidneys of the mice were examined histologically to determine the levels of organ or tissue injuries in the acute toxicity test. Fig. 2 illustrates microscopic inspections of these two organs in both the treated and control groups of mice, with no noticeable differences in the pathological pathways and a regular distribution of stained structures. The stained liver sections showed normal hepatic cell architecture with a well-defined central vein, sinusoids, sheets of hepatocyte,

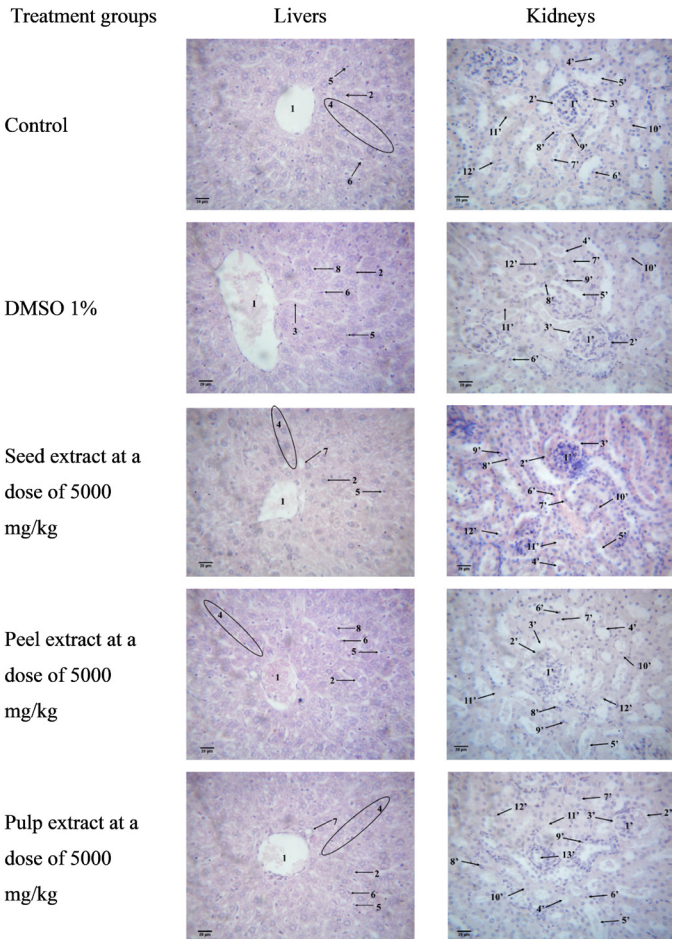

Fig. 2 Histopathology of liver and kidney of mouse groups in acute toxicity test at a dose of 5,000 mg/kg body weight. 1 = central vein; 2 = normal hepatocytes; 3 = sinusoids; 4 = sheets of hepatocyte; 5 = monocytes; 6 = kupffer cell; 7 = bile duct; 8 = binuclear hepatocyte; 1' = malpighi capillaries; 2 = glomerulus; 3' = Bowman's capsule; 4' = proximal convoluted tubule; 5' = distal convoluted tubule; 6' = binuclear cell; 7' = normal cell; 8' and 9' = macrophage cell; 10' = renal interstitium; 11' = necrosis; 12' = pyknotic nucleus; 13' = arteries; DMSO = dimethyl sulfoxide.

monocytes, Kupffer cells, bile duct and binuclear hepatocytes in both groups (control and treatment; data not shown). The stained kidney sections of all groups had normal kidney cell architecture with well-defined Malpighi capillaries, glomerulus, Bowman's capsule, proximal convoluted tubules, distal convoluted tubules, binuclear cells, normal cells, macrophage cells, renal interstitium, pyknotic nuclei and arteries. In general, the liver and kidney cells of both the treated and untreated mice had regular morphological characteristics, with a normal appearance and no tissue rupture.

Conclusion

This study has provided the first published systematic evaluation of the acute oral toxicity of *B. ramiflora* extracts (peel, pulp and seed) in mice. No mortality, behavioral changes or significant alterations in hematological and biochemical parameters were observed at doses up to 5,000 mg/kg, indicating that the extracts were safe under acute exposure. These findings should provide essential baseline data for developing Ha Chau fruit as a potential ingredient in food and health-related products. Future studies should include quantitative analysis of bioactive compounds and screening for possible contaminants such as heavy metals and pesticides, to confirm the fruit's composition and to ensure consumer safety.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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