



Semi-chronic toxicity study of the extracts from different parts of burmese-grape *Baccaurea ramiflora* fruits of Ha Chau variety using mouse model

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Article info.

Received 20 Oct 2022

Revised 24 Oct 2022

Accepted 25 Oct 2022

Keywords

Dau Ha Chau, fruit composition, morphological feature, histopathological study, semi-chronic toxicity

ABSTRACT

This study evaluated the semi-chronic toxicity of seed, peel, and pulp extracts from burmese-grape fruits of Ha Chau variety on Swiss albino mice. The ethanolic extract from peels and seeds as well as fruit juice from squeezing pulp were dried under a vacuum to obtain the crude extracts. The distilled water (control group) or these extracts at a daily dose of 400 mg/kg of body weight were directly inserted into the stomach of mice for 90 days. The mice were observed for toxicity signs, externally morphological features of organs, and histopathology after 45- and 90-days of treatment. The results revealed that there were no toxicity signs and statistically insignificant differences in body weight gain, the ratio of organ weight to body weight, and blood glucose level at 45 days of treatment. At 90 days of treatment, similar results were observed, except that the ratio of kidney weight to body weight significantly increased in peel extract mouse group as compared to the control. The semi-chronic toxicity assessment showed that Ha Chau fruit (HCF) seed and pulp extracts were safe at a daily dose of 400mg/kg for 90 days in mice and that HCF peel extract was safe when orally administered for 45 days.

1. INTRODUCTION

Ha Chau variety was selected from the local varieties of burmese-grape (*Baccaurea ramiflora* Lour.). grown in Phong Dien district, Can Tho city, Viet Nam. Currently, this variety is widely grown in this region. Every year, the fruit harvesting season of this tree is from the 6th to the 10th lunar. HCF is a special fruit that has regional characteristics and a delicious taste. Consequently, it was awarded a trademark in 2006 by the Intellectual Property Office of Viet Nam. The fruits have been mainly consumed as fresh produce, processed food, and medicine. The way to eat the freshly ripe HCF is to remove the peel and swallow the pulp and the seeds because the pulp clings to the seed very tightly.

Sometimes the whole fresh HCF (peel, pulp, and seed) has been used in traditional cooking.

According to (Hossain et al., 2017; Padayatty et al., 2003; Sundriyal & Sundriyal, 2004), ripe *Baccaurea ramiflora* Lour. fruits had a high value of vitamin C, water, carbohydrates, fiber, magnesium, potassium, phosphorus, and iron which are beneficial to human health. While the pulp of *Baccaurea ramiflora* Lour. contained the highest value of total phenolics and total flavonoids, the peel ranked second and the seed had the lowest (Uddin et al., 2018). In addition, *Baccaurea ramiflora* Lour. juice also contains high levels of phenolic and flavonoid compounds that have high antioxidant activity (Goyal et al., 2013; Uddin et al., 2018). Many studies have shown the

potential health benefits of plant polyphenols due to their powerful antioxidant properties that help to prevent chronic diseases related to oxidative stress (Dai & Mumper, 2010).

The medicinal value of *Baccaurea ramiflora* Lour. has been demonstrated by the antiviral properties of the fruit, the diuretic activity of stem bark (Goyal et al., 2013), and the use of the fruit for skin disease treatment (Hasan et al., 2009). The peel of mature fruits was used to produce jellies and jams due to the high content of pectin (Hossain et al., 2017). Moreover, fruits provide energy, vitamins, minerals, dietary fibers, and many bioactive compounds that enhance human health in preventing various cancer and age-related diseases (Dembinska-Kiec et al., 2008; Rekhy & McConchie, 2014).

In the harvesting season, the amount of fruits supplied in the market is very large. Characterized by a pleasant aroma and harmonious sweet and sour taste, this fruit is very attractive for fresh consumption. Therefore, it is possible that a person can consume a large number of fruits at the same time during the ripening season. Currently, there have been no published data on HCF safety and systemic evaluation at high quantities consumed. This study aimed to evaluate the semi-chronic toxicity of the peel, pulp, and seed extracts from HCF by using mouse model.

2. MATERIALS AND METHOD

2.1. Plant collection

Fresh HCF was harvested in the August lunar month from a garden in Phong Dien District, Can Tho City, Viet Nam and transported quickly to the Food Laboratory of Can Tho University, Viet Nam. After rinsing in tap water, the ripe fruits were separated into peels, pulps, and seeds and stored at 4°C for further uses.

2.2. Preparation of the extract

HCF peels were cut into small pieces and dried in hot-air oven (Memmert KS19, Germany) at 50±2°C for 8 h. The seeds were dried in hot-air oven at 50±2°C for 12h. The dry peel and seed were ground into raw powders with a moisture content of 8-10%. 200 g of powder was soaked in 4 L of 96% ethanol for 24 h at room temperature and then filtered through Whatman No. 1 paper. The extraction was repeated three times. The fruit pulp was squeezed to get the juice extract. The filtrate of three extracts (peels, pulps, and seeds) was collected and evaporated by a rotary evaporator (Ika, model RV10

digital V, Germany) at 58±2°C to dryness to obtain 33.21 g of peel extract, 20.91 g of seed extract and pulp extract with the moisture content below 10%. Three extracts were used to evaluate semi-chronic toxicity in mice.

2.3. Approval from animal ethics committee

The study was performed with the approval of the Animal Ethics Committee of Can Tho University, Viet Nam, which approved all experimental protocols (No: DHC2021-02KNN).

2.4. Semi-chronic toxicity assay

Semi-chronic toxicity was conducted according to World Health Organization (Organization, 2008), the method described in the study of Kandimalla et al. (2016), and the Vietnam Health Ministry's regulation with modifications. Healthy male Swiss albino mice, 5-6 weeks old, weighing about 18-22 g were provided by the Institute of Vaccines and Medical Biologicals (IVAC), Viet Nam. They were allowed free access to drinking water and pellet diet (IVAC, Viet Nam). Mice were kept in the experimental facility for 1 week to allow them to acclimate before setting up the experiment.

Before starting the experiment, the mouse's body weight was recorded. Animals (5 mice/group) were randomly assigned to control and treatment groups. The control group received distilled water, while other groups received dimethyl sulfoxide (DMSO) at 1% (v/v) concentration and the extracts (HCF peel, pulp, and seed) at a daily dose of 400 mg/kg body mouse weight, administered orally. Once a day, the same volume of distilled water or extracts was injected into the esophagus of each mouse through the curved needle and syringe for 7 to 9 hours during 90 days. Mice were individually observed for toxicity signs during 90 days of the study. After 45 and 90 days, the body and organs of all animals were weighed (liver, kidney, spleen) weight, and blood glucose levels were measured to be compared with the control group. For the histopathological examination, liver, kidney, and spleen were preserved in 10% formalin.

2.5. Observation of toxicity signs

The signs of toxicity including changes in skin and fur, eyes and mucous membranes, salivation, respiration, urination, diarrhea, activity and behavior, sleep and coma, tremors and convulsions, itchiness, lethargy, and death were recorded during the 90 days of the study. After 45 and 90 days of treatment, mice were weighed before being

sacrificed to determine organ (liver, kidney, spleen) weight and compared with the control group to detect a possible toxic effect of the extracts at the macroscopic level. Body weight gain was calculated using the formula: [(Individual body weight of mouse on sacrifice day (g) - Individual body weight of mouse at the first day of treatment (g))/ Individual body weight of mouse on the first day of treatment] × 100. The ratio of organ weight to body weight (relative organ weight) was calculated by [(Organ weights (g)/ Body weight of mouse on sacrifice day (g)) × 100].

2.6. Evaluate external morphological features of organs for abnormalities

The mouse carcass was placed in dorsal recumbency on a clean dissection board. The skin and the abdominal wall were slit to expose the abdominal viscera. The abdominal wall was widely opened to thoroughly examine and identify the liver, kidney, and spleen in the abdominal cavity. The color changes, size differences, missing or mislocated organs, bleeding or other abnormalities were carefully observed and documented.

2.7. Histopathological study

The liver and kidney were dissected out from the mice of each group and washed with normal saline and immediately fixed in 4% formalin. The organs were dehydrated in gradual grades of ethanol (50–99.5%), cleared in xylene, embedded in paraffin,

and sectioned at 3 μm thickness by the rotary microtome. Sections were processed in alcohol-xylene series, stained with haematoxylin and eosin Y dye, and observed under microscope (Olympus corporation cx21fs1, Tokyo, Japan).

2.8. Statistical analysis

All methods of analysis in this study are done via the Minitab 16 software. The results of all chemical analyses were expressed as mean ± standard deviation. P values less than 0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Observation of toxicity signs

Mice orally treated with 400 mg/kg of HCF seed, peel, and pulp extracts survived the entire experimental period and displayed no signs of discomfort during the 90 days of the study (Table 1). The animals ate food and drank water normally during the study period as compared to the control group. Skin and fur, eyes and mucous membranes, salivation, respiration, urination, diarrhea, activity and behavior, sleep, and coma of extract treated and control mice appeared normal. In addition, there were no signs of tremors and convulsions, itchiness, or lethargy during the 90 days of observation (Table 1). Therefore, the extract of HCF pulps, peels, and seeds fruit had a negligible level of toxicity in mice when administered orally.

Table 1. Behavioral patterns of mice in the control and treated groups during 90 days

Toxicity signs	Control	Treatment groups			
		DMSO 1%	Seed extract	Peel extract	Pulp extract
Skin and fur	N	N	N	N	N
Eyes and mucous membranes	N	N	N	N	N
Salivation	N	N	N	N	N
Respiration	N	N	N	N	N
Urination	N	N	N	N	N
Diarrhea	N	N	N	N	N
Activity and behavior	N	N	N	N	N
Sleep and coma	N	N	N	N	N
Tremors and convulsions	NF	NF	NF	NF	NF
Itchiness	NF	NF	NF	NF	NF
Lethargy	NF	NF	NF	NF	NF
Dead	NF	NF	NF	NF	NF

Key: N = Normal, NF = Not found

3.2. Effects of HCF extracts on organ-to-body weight ratios and blood glucose level of mice after 45 days of treatment

Regardless of the body weight changes and relative organ weight after 45 days in Table 2, there was a

sharp increase in the body weight in all mice test groups, with the ratio of body weight gain fluctuating from 55,52±2,46 to 72,99±2,45%. The animals given HCF seed extract got the highest ratio result in body weight gain, each with 70,93±8,93%,

whereas the opposite was true for the peel extract group (55,52±2,46%). The mice drinking pulp extract came second with 63,93±4,85%. The reason may be that *Baccaurea ramiflora* Lour. The oil content of the seed is 25% (Gogoi, 2017), leading to a dramatic climb in the lipid content in the body, which made the mice gain weight faster than those given other extracts. However, the body weight gains between the control and treated groups were comparable because the differences were not statistically significant.

The other important index of physiological and pathological status in men and animals is organ weight in which the relative organ weight is a useful metric for diagnosing whether an organ has been injured or not (Vaghasiya et al., 2011). According to Wang et al. (2007, an impaired organ often has abnormal tumidity or atrophy. Because of toxicants, the liver, kidney, and spleen are primarily affected

by the metabolic reaction (Dybing et al., 2002). It can be seen from Table 2 that there were no significant differences in the relative organ weights between the treatment and control groups. The ratios of organ weights to body weights fluctuated from 8 to 8,4 (liver weights to body weights), from 1,5to 2,1% (kidney weights to body weights), and from 0,5 to 0,7% (spleens weight to body weights). However, the kidney weight of mice treated with DHC peel extract significantly increased. This caused a climb in the ratio of kidney weights to body weights in mice.

In addition, the blood glucose level is shown in Table 2. Generally, the three types of extracts at a dose of 400 mg/kg did not have any significant effect on the blood glucose level of all tested mice after 45 days of treatment as compared to the control group.

Table 2. Effects on organ-to-body weight ratios and blood glucose level in mice of the control and treated groups after 45 days

Parameters	Control	Treatment groups			
		DMSO 1%	Seed extract	Peel extract	Pulp extract
Body weight gain (%)	72.99 ^a ±2.45	69.09 ^a ±3.67	70.93 ^a ±8.93	55.52 ^b ±2.46	63.93 ^{ab} ±4.85
Ratio of liver weights/ body weights (%)	8.13 ^a ±0.36	8.14 ^a ±0.63	8.36 ^a ±0.50	8.24 ^a ±0.35	7.98 ^a ±0.34
Ratio of kidney weights/ body weights (%)	1.74 ^{ab} ±0.25	1.54 ^b ±0.23	1.97 ^{ab} ±0.36	2.13 ^a ±0.42	1.89 ^{ab} ±0.12
Ratio of spleen weights/ body weights (%)	0.59 ^a ±0.10	0.55 ^a ±0.04	0.52 ^a ±0.13	0.65 ^a ±0.12	0.63 ^a ±0.12
Glucose (mg/dL)	119.52 ^a ±14.77	122.04 ^a ±9.64	123.48 ^a ±2.73	118.08 ^a ±11.07	124.56 ^a ±6.41

Note: different letters in the same row show significant differences at P<0.05.

3.3. Effects of HCF extracts on organ-to-body weight ratios and blood glucose level of mice after 90 days of treatment

When the experimental time was extended to 90 days, the weight of the tested mice continued to rise markedly (Table 3). Body weight gain of the peel extract group was the highest at 125,09±6,20% and that of the seed extract group was the lowest at 118,11±2,92%. Nevertheless, the body weight gains of extract treated and control groups were not

significantly different. Similarly, there were no significant differences in the ratio of liver weights to body weights, ratio of spleen weights to body weights, and blood glucose levels of mice treated with different HCF extracts and control mice. Meanwhile, the ratio of kidney weights to body weights (2,53±0,24%) in the peel extract group was the highest as compared to other groups (p<0.05). It can be concluded that the peel extract at a daily dose of 400 mg/kg can increase the kidney weight of mice after 45 and 90 days of treatment.

Table 3. Effects on organ-to-body weight ratios and blood glucose level in mice of the control and treated groups after 90 days

Parameters	Control	Treatment groups			
		DMSO 1%	Seed extract	Peel extract	Pulp extract
Body weight gain (%)	121.58 ^a ±3.17	122.29 ^a ±1.51	118.11 ^a ±2.92	125.09 ^a ±6.20	120.50 ^a ±5.95
Ratio of liver weights/ body weights (%)	8.00 ^a ±0.39	8.12 ^a ±0.70	8.23 ^a ±0.24	8.62 ^a ±0.18	8.31 ^a ±0.29
Ratio of kidney weights/ body weights (%)	1.71 ^b ±0.21	1.97 ^b ±0.21	1.89 ^b ±0.16	2.53 ^a ±0.24	1.85 ^b ±0.13
Ratio of spleen weights/ body weights (%)	0.61 ^a ±0.08	0.64 ^a ±0.03	0.62 ^a ±0.02	0.65 ^a ±0.02	0.62 ^a ±0.02
Glucose (mg/dL)	121.32 ^a ±6.56	120.24 ^a ±13.49	124.92 ^a ±4.14	120.60 ^a ±12.14	128.16 ^a ±7.36

Note: different letters in the same row show significant differences at P<0.05.

3.4. Morphological features of mouse organs under the effect of HCF extracts

The morphology of the liver, kidney, and spleen of mice at 45 and 90 days of treatment is presented in Figure 1. It can be seen that the external morphology of livers, kidneys, and spleens in mice treated with HCF extracts and control mice did not change. The

liver surfaces in all groups were smooth and with clearly divided lobes. The kidneys in all groups were balanced in size, had a smooth surface, and no abnormal signs. The spleens were moderate in size, without edema or hypertrophy, uniform in color, and had smooth surfaces. Therefore, HCF extracts did not affect the external morphological features of those abdominal organs.

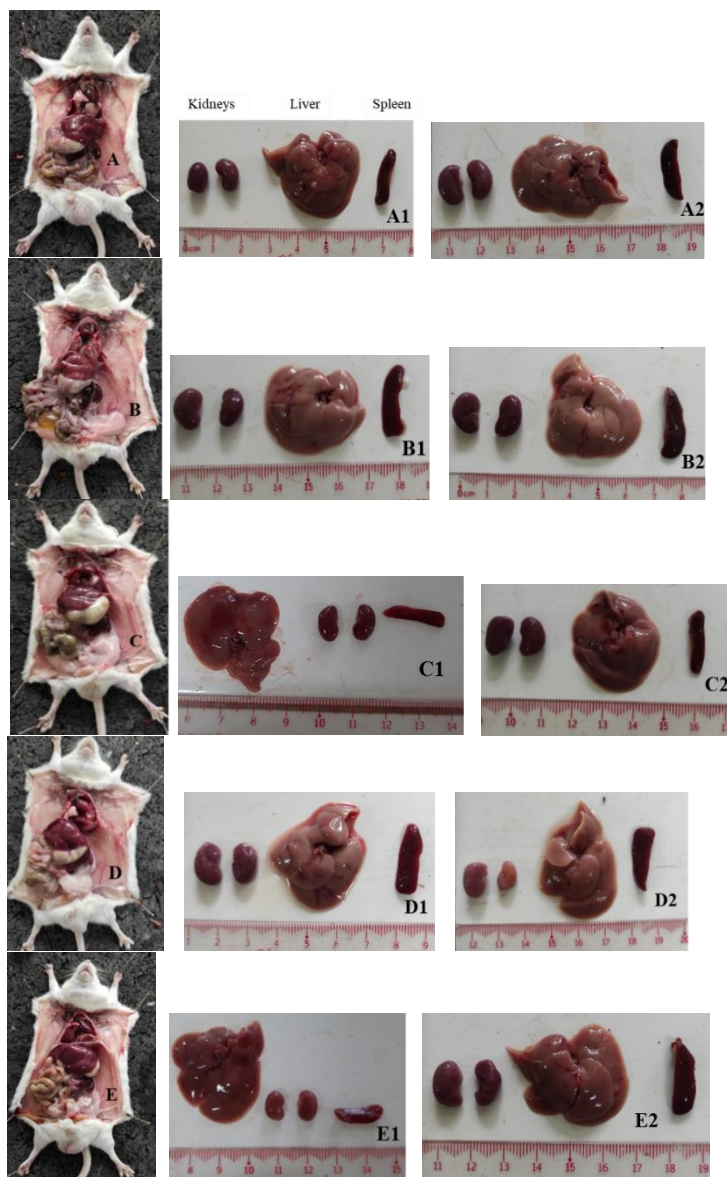


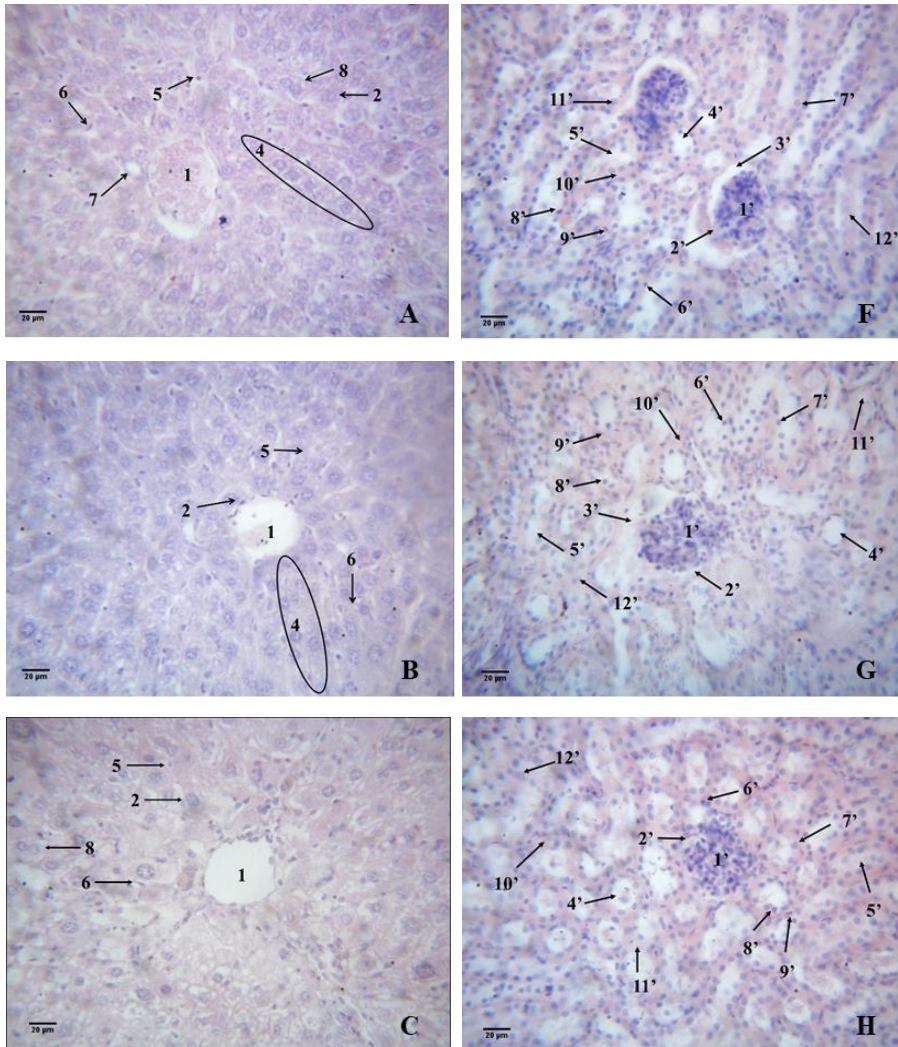
Figure 1. Abdominal organs in the mouse with kidney, liver, and spleen samples isolated from the mouse groups

(A) Abdominal cavity of the control group; (B) Abdominal cavity of the treatment group with DMSO 1%; (C) Abdominal cavity of the treatment group with HCF seed extract; (D) Abdominal cavity of the treatment group with HCF peel extract; (E) Abdominal cavity of the treatment group with HCF pulp extract kidney; (A1), (B1), (C1), (D1) and (E1) Kidney, liver and spleen at Day 45; (A2), (B2), (C2), (D2) and (E2) Kidney, liver and spleen at Day 90.

3.5. Histopathological study

The liver and kidneys are the key organs of primary metabolic targets in excreting waste products of metabolism (drugs and their metabolites) to stabilize the state of the body (Nguenang et al., 2020; Rui, 2014). As a result of the kidney's exposure to toxic substances, the renal tubules could be injured (Bayomy et al., 2017). The histopathological study was performed on the liver and kidneys of mice to

assess the extent of damage to these organs or tissues in a semi-chronic toxicity test. Microscopic observations showed no significant changes in pathological pathways in all organs in the control and treated groups (Figure 2). The mouse liver and kidney cells showed regular morphological structures and normal anatomical appearance, with no sign of necrosis, bleeding or tissue rupture. The stained structures in the cells had regular distribution.



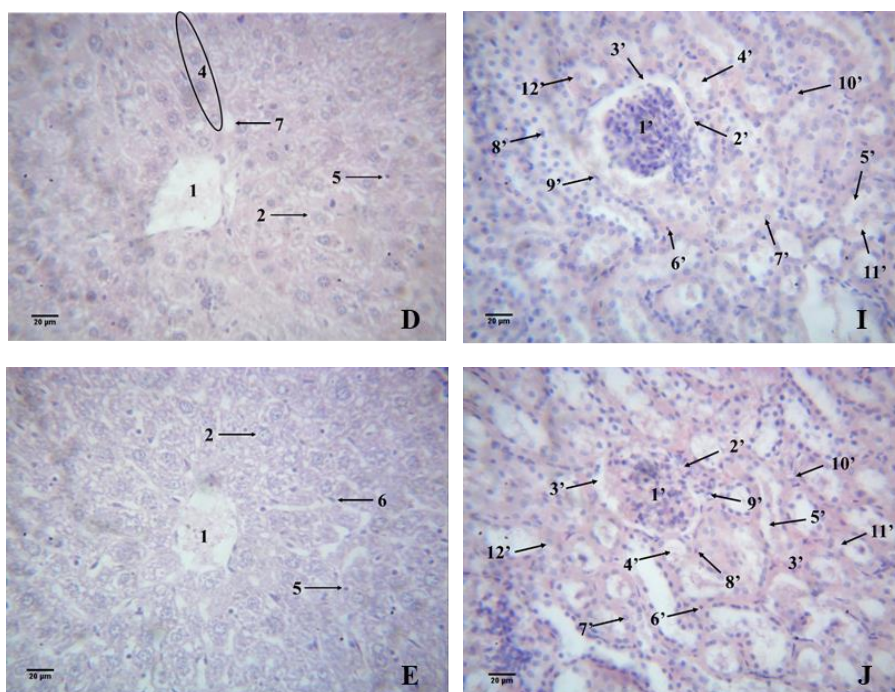


Figure 2. Histopathology of the liver and kidney of mice in semi-chronic toxicity test at Day 90

(A) The liver of the control group; (B) The liver of the treatment group with DMSO 1%; (C) The liver of the treatment group with HCF seed extract; (D) The liver of the treatment group with HCF peel extract; (E) The liver of treatment group with HCF pulp extract; (1) Central vein, (2) Normal hepatocytes, (3) Sinusoids, (4) Sheets of hepatocyte, (5) Monocytes, (6) Kupffer cell, (7) Bile duct, (8) Binuclear hepatocyte).

(F) The kidney of the control group; (G) The kidney of the treatment group with DMSO 1%; (H) The kidney of the treatment group with HCF seed extract; (I) The kidney of the treatment group with HCF peel extract; (J) The kidney of treatment group with HCF pulp extract; (1') Malpighi capillaries, (2') Glomerulus, (3') Bowman, (4') Proximal convoluted tubule, (5') Distal convoluted tubule, (6')

4. CONCLUSION

Ha Chau fruit might be a prized fruit because of its nutritional value. In the semi-chronic toxicity testing, the extracts of seed, peel, and pulp from HCF fruit were safe when administered orally to mice at the dose of 400 mg/kg of body weight for 90 days. There were no adverse effects in behavioral patterns, organ-to-body weight ratios, blood glucose level, external morphological features of organs, and histopathological study. However, the phenomenon of mice kidney weight increasing after

90 days at 400 mg/kg of HCF peel extract should be further evaluated to assess more precisely which quantity consumed and for how long can cause this effect to ensure the safety of this kind of fruit.

ACKNOWLEDGMENT

Nguyen Hong Xuan was funded by Vingroup JSC and supported by the Master, Ph.D. Scholarship Programme of Vingroup Innovation Foundation (VINIF), Institute of Big Data, code: VINIF.2021.TS.154.

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