

Investigation of acute, sub-chronic toxicity, effects of mangiferin and mangiferin solid dispersion (HPTR) on Triton WR1339-induced hyperlipidemia on Swiss albino mice

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Abstract

Mangiferin is a xanthonoid found in Mango leaves in abundance with many effects as a hypoglycemic, antioxidant, and anti-inflammatory agent, plant metabolite, and so on. However, nowadays, mango leaves are merely a waste product in Vietnam. To take advantage of this valuable medicinal resource, extraction conditions of mangiferin using classical and ultrasound methods were researched, and mangiferin was purified from Cat Chu mango leaves (*Mangifera indica* L., Anacardiaceae) collected in Dong Thap. Ultrasound-assisted extraction method was conducted with the following conditions and mangiferin was extracted at a percentage of 6.728% with a purity of 91.11%. Purified mangiferin was evaluated using molecular absorption spectroscopy UV-Vis, scanning electron microscopy (SEM), X-ray diffraction (XRD) spectroscopy, simultaneous thermal analysis (STA: TGA/DSC), and dissolution measurement method. To optimize the solubility and permeability of mangiferin, the solid dispersion system (HPTR) was made by the combination of HPMC 6M:mangiferin at the ratio of 1:5. To investigate the acute, sub-chronic toxicity and hypolipidemia effect of HPTR as compared to purified mangiferin, we followed guidelines for preclinical and clinical trials of Traditional Medicine and Herbal Medicines by the Vietnam Ministry of Health and OECD, and used tyloxapol (Triton WR1339, 400 mg/kg, i.p.) to induce hyperlipidemia. Our results indicated that purified mangiferin and HPTR extract showed no acute toxicity and sub-chronic toxicity and has potential as an antihyperlipidemic agent. The HPTR brought about a significant decrease in total cholesterol, triglycerides and LDL-c when compared to mangiferin, however there was no significance between them.

Keywords

Cat Chu mango leaves, cholesterol, HPTR, mangiferin, tyloxapol, xanthone glycoside

Introduction

In recent years, the use of medicinal herbs has been increasing. Vietnam is a country with a wide diversity of climate and soil, which results in a wealth of biological resources including many plant species with high medicinal value (Thang et al. 2022; Hung et al. 2023b). One of those is the Cat Chu mango tree (*Mangifera indica* L., Anacardiaceae). Cat Chu mango is a popular fruit-bearing tree grown in many places, especially in Dong Thap province. According to statistics from the Dong Thap Department of Agriculture and Rural Development, by March 2023, the total mango-growing area in Dong Thap was more than 14000 hectares (Dong Thap Portal 2023).

Currently, Mango is mainly grown as a fruit-bearing tree. Other parts of Mango, especially the leaves, are merely by-products and often discarded during the growing process. According to many studies, Mango leaves contain many active ingredients such as mangiferin (MGF). MGF is a xanthone glycoside proved to have many pharmacological effects including antidiabetic, lipometabolism regulating, anti-cancer, antioxidant, anti-herpes virus, etc (Mei et al. 2023). Therefore, this study aimed to determine the extraction and purification conditions to maximize the mangiferin content, which just a few studies in Vietnam have published. The results are the basis for application in production practices to take advantage of by-product Mango leaves to generate income for Mango growers and reduce environmental pollution. In addition, mangiferin was extracted with high content and various pharmacological effects, contributing to community health care.

Mangiferin is a C-glucosyl xanthone, which has been shown to have many pharmacological effects including antidiabetic, antitumor, lipometabolism-regulating, antioxidant, analgesic, antibacterial, antiviral, and immunomodulatory effects (Du et al. 2018). Among these effects, antidiabetic and lipometabolism-regulating ones are being researched for clinical application. According to the Ministry of Health, in 2021, in Vietnam, more than 55% of patients with diabetes had complications, of which 34% had cardiovascular complications, 39.5% had ophthalmic and neurological complications, and 24% had renal complications (Ministry of Health 2022). Besides, deaths from cardiovascular disease have been increasing. Mangiferin is considered as a potential for treatment, however, its low solubility and permeability have been a hindrance to oral bioavailability enhancement (Barakat et al. 2022). Therefore, improving the solubility of mangiferin is necessary to increase treatment effectiveness. Several methods for improving solubility have been implemented such as nanoparticle generation (Razura-Carmona et al. 2019), solid dispersion (Nagarani and Gadela 2023), soya phospholipid-mangiferin complex (Bhattacharyya et al. 2014), and so forth. The solid dispersion preparation method is often chosen among the above methods due to its higher drug-carrying rate, simplicity, ease of implementation, and suitability with practical laboratory conditions (Das et al. 2011).

By using hydrophilic polymers as carriers combined with reducing particle size or changing the shape, the mangiferin solid dispersion system improves solubility, thereby enhancing oral bioavailability.

Materials and methods

Extraction procedure

Cat Chu mango leaves (*Mangifera indica* L., Anacardiaceae) that were dark green and thick without pests, damage, and termites were harvested in November 2022 in Cao Lanh, Dong Thap. After being harvested, the leaves were washed and dried at 50–60 °C until the weight remained. Then they were ground and sieved into crude powder that was stored in a sealed package away from moisture.

The research was conducted at the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy and at the Central Laboratory for Analysis, Ho Chi Minh City University of Science.

Survey of extraction conditions using the classical extraction method

Approximately 10 g of dried mango leaf medicinal powder (A) was weighed and put in a conical flask with a stopper. Ethanol (B) was added according to a ratio of (C), then water bath extraction was conducted at a room temperature of (D) for (E) hours (Table 1). Next, the extract was filtered and heated in a water bath at 60 °C to a moisture content of < 20%, then it was dried at a temperature of 50 °C to a moisture content of < 5%. Each sample was extracted 3 times to take the average value.

Survey of extraction conditions using ultrasonic extraction method

Approximately 10 g of dried mango leaf medicinal powder (A) was weighed and put in a conical flask with a stopper. Ethanol (F) was added according to a ratio of (G), then extraction with ultrasound was conducted at a normal temperature for (H) minutes with an amplitude of (M) (Table 1). The extract was filtered and heated similarly to section 2.2.1. Each sample was extracted 3 times to take the average value.

Mangiferin content was evaluated using molecular absorption spectroscopy UV-Vis (this study referred to the research by Nguyen Thi Truc Loan (Truc Loan 2018) and the method was qualified by other research at the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy) at wavelength of 258 nm with a standard concentration of 16 µg/mL, blank sample (ethanol 96%/pH 1.2 buffer (1/9)). Linear line $\hat{y} = 0.0686x - 0.0162$ with correlation coefficient $R^2 = 0.9999$ (> 0.998) in the concentration range of 4.8–16 µg/mL.

Table 1. Survey of extraction conditions using classical and ultrasonic extraction methods.

No	Classical extraction					Ultrasonic extraction				
	Symbol	Ethanol concentration (%) (B)	Ratio of herbs/solvent (C)	Temperature (D) (Turner et al.)	Extraction time (E) (hour)	Symbol	Ethanol concentration (%) (F)	Ratio of herbs/solvent (G)	Ultrasound time (H) (minutes)	Amplitude of ultrasonic waves (M) (%)
1	B1	50	1/12	Room temperature	9	F1	50	1/12	15	70
2	B2	60	1/12	Room temperature	9	F2	60	1/12	15	70
3	B3	70	1/12	Room temperature	9	F3	70	1/12	15	70
4	B4	80	1/12	Room temperature	9	F4	80	1/12	15	70
5	C1	B	1/10	Room temperature	9	G1	F	1/10	15	70
6	C2	B	1/12	Room temperature	9	G2	F	1/12	15	70
7	C3	B	1/15	Room temperature	9	G3	F	1/15	15	70
8	C4	B	1/17	Room temperature	9	G4	F	1/17	15	70
9	D1	B	C	Room temperature	9	H1	F	G	5	70
10	D2	B	C	40	9	H2	F	G	10	70
11	D3	B	C	50	9	H3	F	G	15	70
12	D4	B	C	60	9	H4	F	G	20	70
13	E1	B	C	D	6	M1	F	G	H	60
14	E2	B	C	D	9	M2	F	G	H	70
15	E3	B	C	D	18	M3	F	G	H	80
16	E4	B	C	D	24	M4	F	G	H	90

The crude extract efficiency obtained was calculated according to the following formula:

$$H\% = \frac{m-(m \times n)}{10} \times 100 \quad (\text{Eq. 1})$$

m was the weight of the concentrated extract obtained (g); n was the moisture content of the extract (%).

The mangiferin content in the extracted sample was worked out using the following formula (Bag et al. 2015):

$$X \text{ (mg/g)} = \frac{c \times V}{m} \times n \times H \quad (\text{Eq. 2})$$

c was the value of the mangiferin standard curve (mg); V was the volume of the extract solution (mL); m was the weight of extract in the volume (g/mL); n was the moisture content of the extract (%); H was the extract efficiency (%).

Purification of mangiferin

Survey of solvents of liquid-liquid extraction

240 g of medicinal powder was taken to extract with the best extraction conditions surveyed above, and then purification of mangiferin was investigated as follows:

The crude extract and distilled water were mixed at a ratio of 1:2 (v/v). The liquid-liquid extraction was surveyed with a solution containing mangiferin with each solvent including n-butanol, chloroform, benzene, and ethyl acetate at a ratio of 1:1 (v/v). The lower layer was taken. It was left for about 24 hours for the crystallization of mangiferin, then it was filtered and dried at 50 °C to a moisture content < 5% (Wei et al. 2018; Loan et al. 2021).

Each sample was repeated 3 times to take the average result. The efficiency of purified mangiferin obtained was calculated as the following formula:

$$H\% = \frac{M-(M \times N)}{240} \times 100 \quad (\text{Eq. 3})$$

M was the purified mangiferin weight obtained; N was the moisture content of mangiferin.

The purity of the mangiferin obtained was compared to the standard mangiferin and worked out as follows (Loan et al. 2021):

$$Y (\%) = \frac{A_2 \times C_1}{A_1 \times C_2} \times 100 \quad (\text{Eq. 4})$$

C₁, A₁: concentration and absorbance of standard mangiferin; C₂, A₂: concentration and absorbance of purified mangiferin obtained.

Analysis of mangiferin properties after purification

Appearance: the sample was observed with the naked eye under natural light, and then compared with the data in Vietnamese Pharmacopoeia V (Ministry of Health 2017).

UV-VIS spectrum measurement: the spectrum of the purified mangiferin was compared to the standard by UV-Vis from wavelength 200 nm to 600 nm.

Particle size: the sample was observed using scanning electron microscope (SEM). The test was conducted on a scanning electron microscope JSM-IT 200 (Jeol, Japan). The sample was coated with platinum before analysis with carbon glue to adhere the sample to the copper substrate and a voltage of 10 kV.

Crystal structure: the sample was observed using X-ray diffraction (XRD) spectroscopy. The test was conducted on a D8 Advance Eco device (Bruker, Germany) at a voltage of 40 kV, an amperage of 25 mA, a Cu K-alpha wavelength of 0.154 nm, a step size of 0.02°, and a step time of 0.1s.

Hydration: the experiment was conducted on PT 1600 equipment (Linseis, Germany) by thermal gravimetric analysis (STA: TGA/DSC). The sample was weighed on a 5-digit weighing device (OHAUS Explorer125, USA) in an alumina crucible and stabilized in the analysis chamber

for 10 minutes before the test. The air flow rate was 4 L/hour. It depended on the situation that an inert gas (Argon) or a dry air (DryAir: N₂/O₂ ratio 4:1) would be used. The heating rate was 10 °C/minute.

Dissolution: dissolution was measured by a paddle-type dissolution apparatus Pharmatest PTWS 120D with a rotation speed of 100 rpm at a temperature of 37 ± 0.5 °C. 500 mL of a pH 1.2 dissolution medium (buffer solution was prepared according to section 2.3 of Appendix 2 in Vietnamese Pharmacopoeia V). 80 mg of mangiferin sample was added into the dissolution medium. After 5, 15, 30, 45, and 60 minutes, 10 mL of solution was drawn and filtered through filter papers. 1 mL of the filtrate was drawn and put into a 10 mL volumetric flask, and then a pH 1.2 buffer was added to fit the volume. Absorbance was measured by using molecular absorption spectroscopy UV-VIS at a wavelength of 258 nm.

The blank sample was a pH 1.2 buffer. Each sample was tested 3 times to get the average result. Uncorrected mangiferin concentration at the n^{th} time was calculated as follows:

$$C_n = C_{n0} + \frac{V_0}{V} \times C_{n-1} \quad (\text{Eq. 5})$$

C_n was the corrected concentration at the n^{th} time (μg/mL); C_{n0} was the uncorrected concentration at the n^{th} time (μg/mL); C_{n-1} was the corrected concentration at the $(n-1)^{\text{th}}$ time (μg/mL); V_0 was the volume of the solution withdrawn ($V_0 = 10$ mL); V was the volume of the dissolution medium ($V = 500$ mL).

The percentage of dissolved mangiferin at the time t was calculated according to the formula:

$$\% \text{mangiferin} = \frac{C_n \times 500}{m \times 1000} \times 100 \quad (\text{Eq. 6})$$

n was the corrected concentration at the n^{th} time (μg/mL); m was the mangiferin content in the sample (mg).

The percentage of dissolved purified mangiferin at the time t was calculated and compared with the dissolution of the control mangiferin according to the independent model with the similarity factor f_2 :

$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{i=1}^n (R_i - T_i)^2 \right)^{-0.5} \times 100 \right\} \quad (\text{Eq. 7})$$

n was the number of times of sampling R ; R_i was the dissolution of the reference substance at the time t ; T_i was the dissolution of the test substance at the time t ; $0 < f_2 < 50$: two lines represented different dissolution profiles.

Preparation of HPTR

The mangiferin solid dispersion system combined with HPMC 6M at the ratio of 1:5 ratio to make the preparation helped improved the solubility of the mangiferin solid dispersion system.

Experimental animals

All animals were treated in accordance with the Institute for Laboratory Animal Research (ILAR) Guidelines

for the Care and Use of Laboratory Animals (Council 2011). All the experiments were conducted in Laboratory Animal House, Department of Pharmacology, Can Tho Medicine and Pharmacy University, Can Tho and the experimental study (Protocol No.2313/QĐ-CTUMP) was approved by the Institutional Council Reviews Committee of Can Tho Medicine and Pharmacy University, Can Tho.

Eight-week-old Swiss albino mice in both sexes were obtained from Pasteur Institute, Ho Chi Minh City, Vietnam, each weighing approximately 20–25 g. Then, they were randomized, and housed in a temperature-controlled animal facility (24 ± 2 °C) and fed *ad libitum*. Mice were maintained for at least 7 days before starting the experiments.

Acute toxicity

Female and male mice were used for the acute and sub-chronic oral toxicity tests. 16 mice (8 males and 8 females) were used in an acute oral toxicity test, whereas 24 mice (12 males and 12 females) were used in sub-chronic oral toxicity test (the repeated dose 28 day). The toxicity tests were carried out according to the Guidelines for preclinical and clinical trials of Traditional Medicine and Herbal Medicines by the Vietnam Ministry of Health (Ministry of Health 2015) and OECD (OECD Rome 2001; Hung et al. 2023a) with slight modifications. Prior to the start of the experiment, the body weight of animals was recorded individually for calculating the proper treatment dosage. The volume was adjusted depending on the body weight of the mice using 10–20 mL/kg as this is the normal volume to be used in the mice as mentioned elsewhere (Turner et al. 2011). The mice were nulliparous and non-pregnant. They were acclimatized to laboratory conditions for 7 days before the experiments and housed in groups of three for acute oral toxicity test and in groups of four for sub-chronic oral toxicity test.

Sub-chronic toxicity

24 mice were randomly divided into three groups of 8 mice each ($n=8$ /group, 4 males and 4 females) as mentioned elsewhere (OECD 2008; Ramaswamy et al. 2012; Ministry of Health 2015). Three groups were administered daily using oral gavage for 28 consecutive days.

Group 1 received distilled water and served as control.

Groups 2, and 3 received HPTR 200 mg/kg and mangiferin 200 mg/kg body weight, respectively.

Mice were observed for 28 days and anesthetized following a 12-hour fast. Blood samples were drawn from the heart into a vacuum tube, and organs (liver, kidneys) were collected for hematoxylin and eosin staining. Mortality and general toxicity signs of the animals were monitored and recorded daily throughout the study. The body weight of all the groups had been recorded before treatment and at the end of each week (OECD 2008; Ministry of Health 2015).

Experimental design to investigate the effect of HPTR and mangiferin on acute hyperlipidemia

Mice received a single dose of tyloxapol (Triton WR1339) [400 mg/kg, intraperitoneal injection (i.p.); T0307, Sigma-Aldrich, MO, USA] (Huynh et al. 2024) and were sacrificed 1 day later to investigate the hypolipidemic effect of HPTR and mangiferin on tyloxapol-induced acute hyperlipidemia in mice. Tyloxapol was dissolved in 0.9% saline. Mice received the first dose of HPTR and mangiferin (200 mg/kg, p.o.) one day before the dose of Triton WR1339 and the second dose after Triton WR1339 administration. Mice were sacrificed following a 12-hour fast, blood samples were collected from the heart. Freshly collected blood samples were centrifuged at 4000 rpm for 20 minutes at room temperature, and serum was collected as the supernatant for total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) measurements.

Serum chemistry analysis for sub-chronic toxicity

Blood samples were kept in K2EDTA tube for analyzing hematological parameters [red blood cell (RBC), hemoglobin (Hb), white blood cell (WBC), mean corpuscular hemoglobin (MCH), platelet (PLT), mean corpuscular volume (MCV), hematocrit (HCT)] and in plain tubes for biochemical parameters [urea, creatinine, alanine aminotransferase (Ministry of Health 2015) and aspartate aminotransferase (Singh et al. 2016)].

Hematoxylin & eosin (H&E) staining

Livers and kidneys were collected and fixed by overnight immersion in 10% buffered formalin. According to a standard protocol, paraffin-embedded specimens were prepared, and 5.0 μm sections were stained with H&E (Sigma-Aldrich, St. Louis, MO, USA). Hepatic pathology was assessed with an Olympus BX40 microscope by an investigator who was blinded to the experimental treatment groups (Mai et al. 2019).

Biochemical parameters and lipid profile measurements

The measurement procedure was conducted following the manufacturer's protocol [Cholesterol (03039773190, Cobas, Germany), Triglyceride (20767107322, Cobas, Germany), HDL-c (07528566190, Cobas, Germany), LDL-c (07005717190, Cobas, Germany), Creatinine (04810716190, Cobas, Germany), Urea (04460715190, Cobas, Germany), ALT (20764957322, Cobas, Germany), and AST (20764949322, Cobas, Germany)].

Statistical analysis

Statistical analyses were performed using IBM SPSS 20 software. One-way analysis of variance (ANOVAs) was used to examine the main effects. To determine specific group differences in case of significant main effects, the one-way ANOVA was followed by Tukey post-hoc test. P-values of less than 0.05 were deemed to indicate statistical significance.

Results

Survey of extraction conditions using classical and ultrasonic extraction methods

The results of extraction efficiency and mangiferin content obtained under different conditions are presented in Tables 2, 3. The mangiferin content was the most optimal when extracted using the classical method with the parameters: ethanol 60% (B2), herb/solvent ratio of 1/15 (C3), temperature of 60 °C (D4), and extraction time of 9 hours (E2).

The mangiferin content obtained was the most optimal using the ultrasound-assisted extraction method with the following parameters: ethanol 70% (F3), medicinal material/solvent ratio of 1/15 (G3), ultrasound time of 20 minutes (H4), and ultrasound amplitude of 70% (M2).

The comparison of E2 and M2 formulas showed that the efficiency and mangiferin content obtained by the ultrasonic extraction method were higher than by the classical extraction one. Purification of mangiferin

Table 2. Extraction efficiency and mangiferin content obtained by the classical extraction method.

Formula	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4	E1	E2	E3	E4
1 st H%	4.65	4.48	4.46	4.40	4.74	4.73	4.77	4.68	4.70	4.61	4.71	4.74	4.72	4.70	4.81	4.67
2 nd H%	4.49	4.67	4.44	4.58	4.57	4.64	4.58	4.58	4.82	4.71	4.72	4.70	4.75	4.82	4.83	4.78
3 rd H%	4.44	4.52	4.65	4.54	4.53	4.52	4.58	4.63	4.58	4.81	4.72	4.78	4.78	4.76	4.67	4.89
Average H%	4.53	4.56	4.52	4.51	4.61	4.63	4.64	4.63	4.70	4.71	4.72	4.74	4.75	4.76	4.77	4.78
RSD	2.48	2.23	2.63	2.10	2.52	2.20	2.43	1.02	2.53	2.17	0.11	0.78	0.63	1.30	1.82	2.32
X 1	9.60	11.1	8.99	8.47	8.37	11.12	12.52	10.18	12.93	19.75	21.12	22.62	20.52	23.25	23.93	24.24
X 2	9.65	10.3	8.76	8.67	8.37	11.44	12.78	10.39	12.75	19.22	21.83	22.96	20.34	23.79	23.05	23.45
X 3	9.27	10.9	8.91	8.57	8.04	11.25	13.16	10.62	13.27	20.13	20.81	23.77	19.73	22.58	23.95	24.72
Average X	9.51	11.1	8.89	8.57	8.26	11.27	12.82	10.40	12.98	19.70	21.25	23.12	20.20	23.21	23.64	24.14
RSD	2.21	2.04	1.33	3.50	2.33	2.49	2.51	2.12	2.03	2.32	2.46	2.56	2.05	2.61	2.17	2.66

Table 3. Extraction efficiency and mangiferin content obtained by ultrasonic extraction method.

Formula	F1	F2	F3	F4	G1	G2	G3	G4	H1	H2	H3	H4	M1	M2	M3	M4
1 st H%	6.12	6.32	6.28	6.25	6.08	6.26	6.21	6.04	6.12	6.38	6.24	6.14	6.28	6.22	6.37	6.16
2 nd H%	6.18	6.10	6.24	5.97	6.11	6.26	6.25	6.37	6.41	6.18	6.23	6.42	6.38	6.37	6.47	6.45
3 rd H%	5.97	6.02	6.05	6.26	6.27	5.98	6.17	6.22	6.25	6.23	6.35	6.26	6.21	6.24	6.14	6.32
Average H%	6.09	6.15	6.19	6.16	6.15	6.16	6.21	6.21	6.26	6.26	6.27	6.27	6.28	6.28	6.33	6.31
RSD	1.82	2.49	2.03	2.63	1.47	2.61	0.69	2.61	2.32	1.68	1.05	2.26	0.99	1.29	2.67	2.27
X 1	31.41	31.31	33.78	31.18	28.01	33.23	35.02	34.03	29.72	31.75	34.96	37.51	33.31	37.62	34.99	35.69
X 2	30.44	31.71	34.76	32.86	27.06	33.26	36.06	35.22	30.36	33.34	33.76	37.09	33.79	37.02	35.78	35.51
X 3	30.14	32.26	33.17	31.82	28.15	34.75	36.86	34.30	28.78	32.06	34.31	38.49	34.73	38.55	34.43	34.03
Average X	30.66	31.76	33.90	31.95	27.74	33.75	35.98	34.52	29.62	32.38	34.35	37.70	33.94	37.73	35.07	35.08
RSD	2.17	1.50	2.37	2.65	2.14	2.58	2.56	1.81	2.68	2.60	1.75	1.91	2.13	2.04	1.93	2.60

Survey of solvents of liquid-liquid extraction

When ethyl acetate was used, many impurities were removed with the highest purity of 91.11% (Table 4). Then, the mangiferin sample purified with ethyl acetate was taken for further analysis.

Table 4. Extraction efficiency and purity of mangiferin obtained after purification.

Solution	N-butanol	Chloroform	Benzene	Ethyl acetate
1 st H%	3.028	4.335	2.210	2.234
2 nd H%	3.154	4.272	2.141	2.316
3 rd H%	3.126	4.205	2.255	2.294
Average H%	3.103	4.271	2.202	2.281
RSD	2.125	2.609	1.512	1.860
1 st purity	30.25	25.89	36.69	91.07
2 nd purity	31.34	27.12	38.30	91.21
3 rd purity	31.63	26.55	37.55	91.05
Average purity	31.07	26.52	37.51	91.11
RSD	2.342	2.321	2.148	0.096

Analysis of mangiferin properties after purification

Appearance: the crystals were pale yellow, smooth, odorless, and consistent with the data in the Vietnamese Pharmacopoeia V (Fig. 1A).

UV-Vis spectrum measurement: the spectrum of the standard mangiferin and the purified mangiferin at the same concentration of 16 µg/mL were completely similar to the full absorption peaks in the Vietnamese Pharmacopoeia V (Fig. 1B).

Particle size: based on the results under the scanning electron microscope, both samples had similar particle sizes (Fig. 2). Primary particles with sizes from 100 to 500 nm were aggregated to form secondary particles with sizes of 5–10 µm.

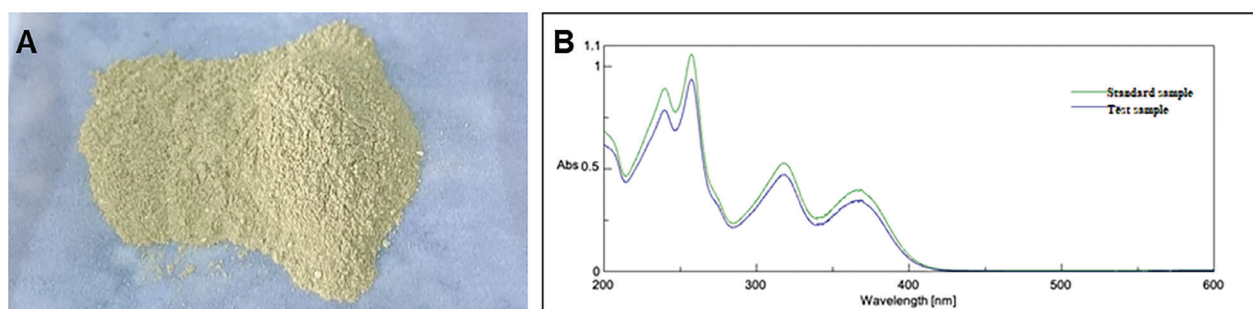
Crystal structure: X-ray powder diffraction results showed that both control mangiferin and purified mangiferin samples were crystalline. However, the purified sample had some additional peaks at positions of 6°–15°. The signal intensity of the purified mangiferin at the main peaks (positions of 10°–30°) was much lower than that of the control mangiferin, showing that the crystallinity of the purified mangiferin was lower (Fig. 3A, B).

Hydration: the moisture content of the control sample was about 2% while the figure for purified mangiferin was 8%. The evaporation of water from the surface took place in the reaction zone from 80 °C to 120 °C.

The number of water molecules in the structure of $C_{19}H_{18}O_{11} \cdot xH_2O$ was estimated. The amount of water in the structure was calculated based on the formula $(18x)/(423 + 18x)$:

- Control sample: 18%; $x = 5$ (Fig. 4A).
- Purified sample: 13%; $x = 4$ (Fig. 4B).

Dissolution: in the first 5 minutes, the dissolution of the purified mangiferin was greater than that of the control mangiferin. However, from 5 minutes onwards, there was a reverse. The level of active ingredient dissolution of both samples tended to increase over time and had a value of $f_2 = 50.17$ (Fig. 4C).

**Figure 1.** A Mangiferin after purification B UV-Vis spectrum of standard mangiferin and purified mangiferin.

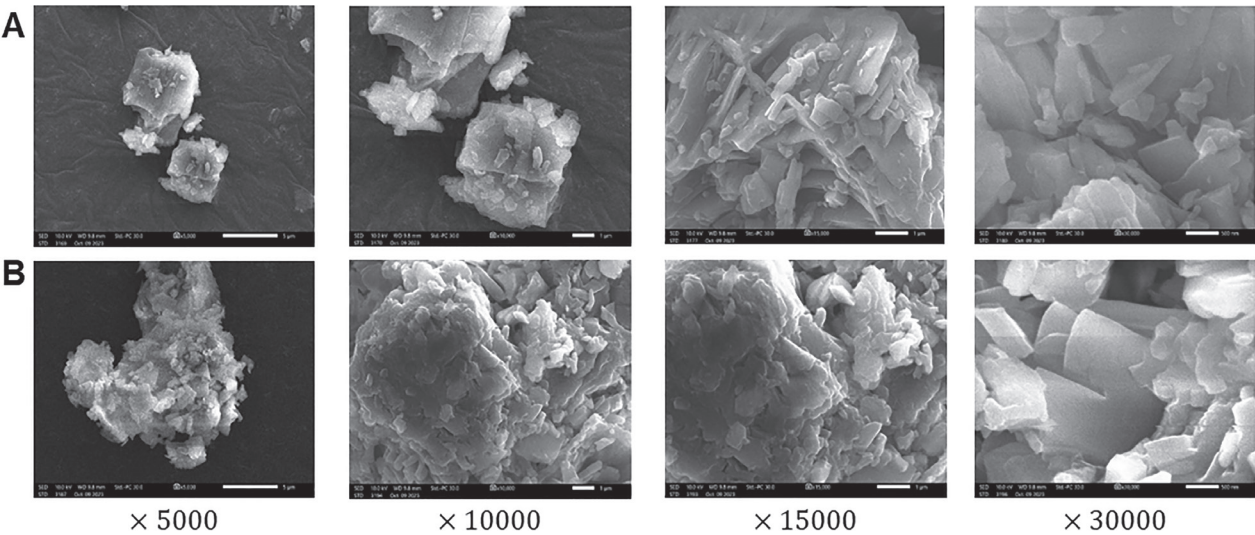


Figure 2. A Control mangiferin and B Purified mangiferin at magnifications of $\times 5000$, $\times 10000$, $\times 15000$, and $\times 30000$.

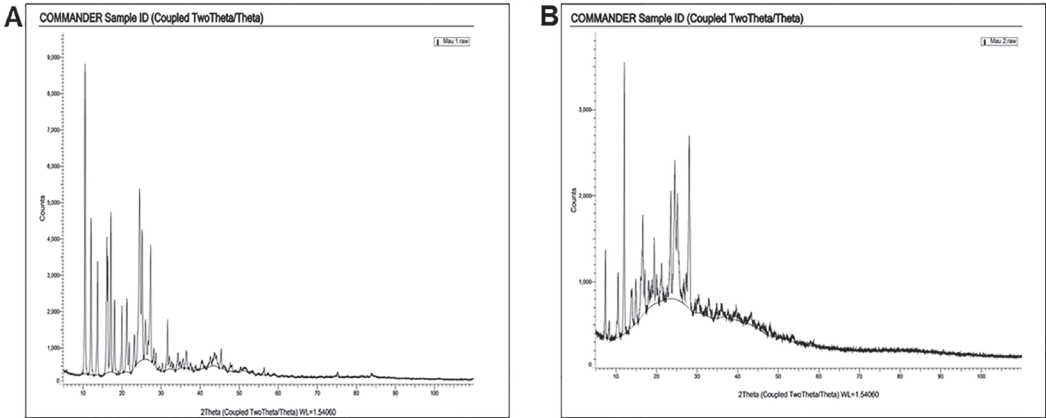


Figure 3. X-ray diffraction spectra of A control mangiferin and B Purified mangiferin.

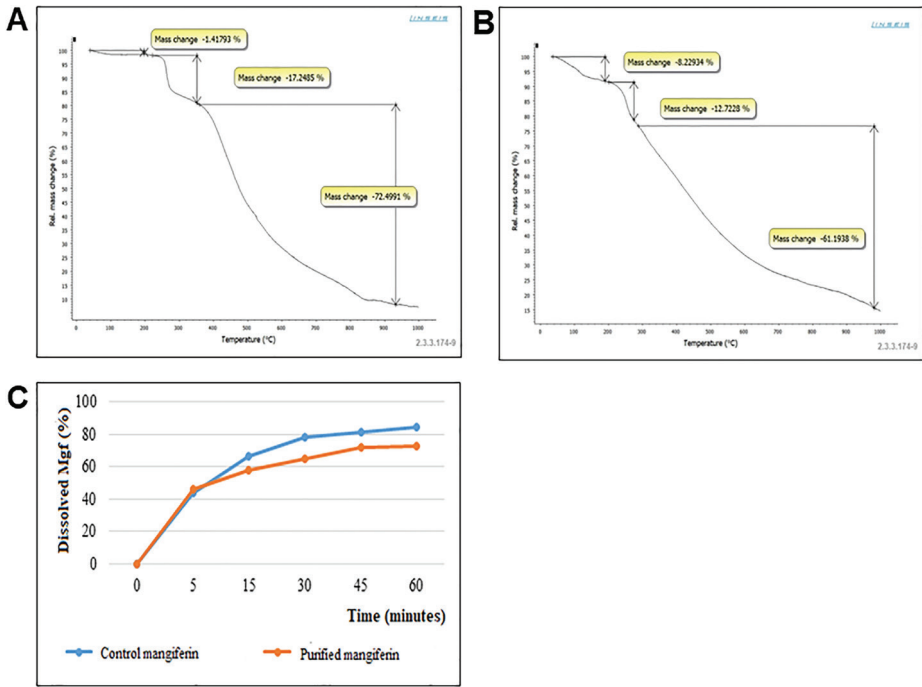


Figure 4. Thermogravimetric analysis diagram of A control mangiferin and B purified mangiferin and C dissolution graph of mangiferin at pH 1.2.

General sign and behavioral analysis

Oral administration of HPTR and mangiferin showed no treatment-related mortality in both sexes of mice for both acute and sub-chronic oral toxicity tests throughout the study. Physical observation of the HPTR (4200 mg/kg, equivalent to mangiferin 700 mg/kg) and mangiferin treated mice for both acute (700 mg/kg) and sub-chronic oral toxicity tests (200 mg/kg) throughout this study indicated that none of them showed signs of toxic effects such as changes in weight, skin and fur, eyes and mucous membrane, behavior pattern, tremors, salivation, and coma. Diarrhea appeared at the rate of 12.5% (200 mg/kg mangiferin-treated group) in the 3rd week to 25% (200 mg/kg HPTR-treated group) in the first week in sub-chronic oral toxicity tests but mice recovered after several days. No mortality and other general toxicity signs of the animals were recorded throughout the study.

No macroscopic or microscopic pathological abnormalities in the livers and kidneys were observed in all groups, (700 mg/kg in acute oral toxicology test and 200 mg/kg in sub-chronic oral toxicity tests for 28 days). According to the Globally Harmonized Classification System, mangiferin and HPTR can be classified as Category 5 and this provides direct relevance for protecting animal's health up to the high dosage used in those studies.

Effect of HPTR and mangiferin on hematological parameters in sub-chronic oral toxicity tests

The hematological profile of control and HPTR/mangiferin treated groups is summarized in Table 5. The results concluded that all hematological parameters such as red blood cell (RBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), white blood cell (WBC), platelet (PLT), mean corpuscular volume (MCV), hematocrit (HCT) showed no significant differences between the groups in sub-chronic toxicity tests ($p > 0.05$).

Table 5. Hematological parameters in sub-chronic oral toxicology of mangiferin and HPTR. Red blood cell (RBC); Hemoglobin (Hb); Hematocrit (HCT); Mean corpuscular volume (MCV); Mean Corpuscular Hemoglobin (MCH); Platelet (PLT), and white blood cell (WBC). Comparison between the groups was made by one way analysis of variance (ANOVA) followed by a Tukey post-hoc test.

Parameters	Control	Mangiferin 200 mg/kg	HPTR 200 mg/kg
RBC (Tera/L)	8.68 ± 0.52	9.89 ± 0.73	9.72 ± 0.667
Hb (g/dL)	12.42 ± 0.62	14.17 ± 0.94	13.76 ± 2.18
HCT (%)	39.55 ± 5.21	43.98 ± 3.21	43.6 ± 4.55
MCV (fL)	48.33 ± 3.33	44.5 ± 2.12	44.82 ± 0.246
MCH (pg)	14.67 ± 1.04	14.34 ± 0.62	14.12 ± 0.15
PLT (G/L)	838.50 ± 136.85	711.28 ± 290.21	743 ± 69.195
WBC (G/L)	8.38 ± 2.37	8.45 ± 0.46	11.5 ± 2.078

Effect of HPTR and mangiferin extract on serum biochemical parameters in sub-chronic oral toxicity tests

The data on biochemical parameters in control and HPTR/mangiferin treated groups of sub-chronic oral toxicity tests are presented in Table 6. The results concluded that all biochemical parameters such as urea, creatinine, alanine aminotransferase and aspartate aminotransferase showed no significant differences between the groups in sub-chronic toxicity tests ($p > 0.05$).

Table 6. Biochemical parameters in sub-chronic oral toxicology of mangiferin and HPTR. Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); urea, and creatinine. Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test.

Parameters	AST (U/L)	ALT (U/L)	Urea (mmol/L)	Creatinine (μmol/L)
Control	84.33 ± 34.33	35.83 ± 7.73	4.72 ± 0.31	30.00 ± 2.48
Mangiferin 200 mg/kg	97.57 ± 24.49	26.86 ± 4.81	4.56 ± 0.65	33.74 ± 8.89
HPTR 200 mg/kg	87.00 ± 23.43	22.00 ± 2.00	3.87 ± 1.17	28.44 ± 1.98

Histopathological observation

The photomicrographs of the liver and kidney of the control and HPTR/mangiferin treated groups, both male and female, showed normal morphological architecture. Similar to the control group, the liver of HPTR/mangiferin treated animals showed normal cellular architecture, binucleation and was without any distortions. Furthermore, signs of injury, necrosis, congestion, fatty acid accumulation, or hemorrhagic regions around the central vein or sinusoids of the liver were not observed (Fig. 5).

For the kidneys, there were no morphological changes for HPTR/mangiferin treated groups. The appearance of the glomerular architecture showed normal structure which was similar to that of the control groups (Fig. 5).

Effects of HPTR and mangiferin in a tyloxapol-induced acute hyperlipidemia mouse model

The total cholesterol (A), triglyceride (B), HDL-c (C) and LDL-c (D) levels of the control, tyloxapol, atorvastatin, mangiferin 200 mg/kg, and HPTR 200 mg/kg treated groups were demonstrated in Fig. 6.

We observed a significant difference in total cholesterol level in mice injected with tyloxapol as compared with the control group ($p < 0.05$). In this study, mangiferin and HPTR at 200 mg/kg and atorvastatin groups reduced blood cholesterol significantly as compared to the tyloxapol group ($p < 0.05$). Specifically, HPTR at 200 mg/kg was as effective as atorvastatin in reducing cholesterol levels compared to the tyloxapol group (Fig. 6A).

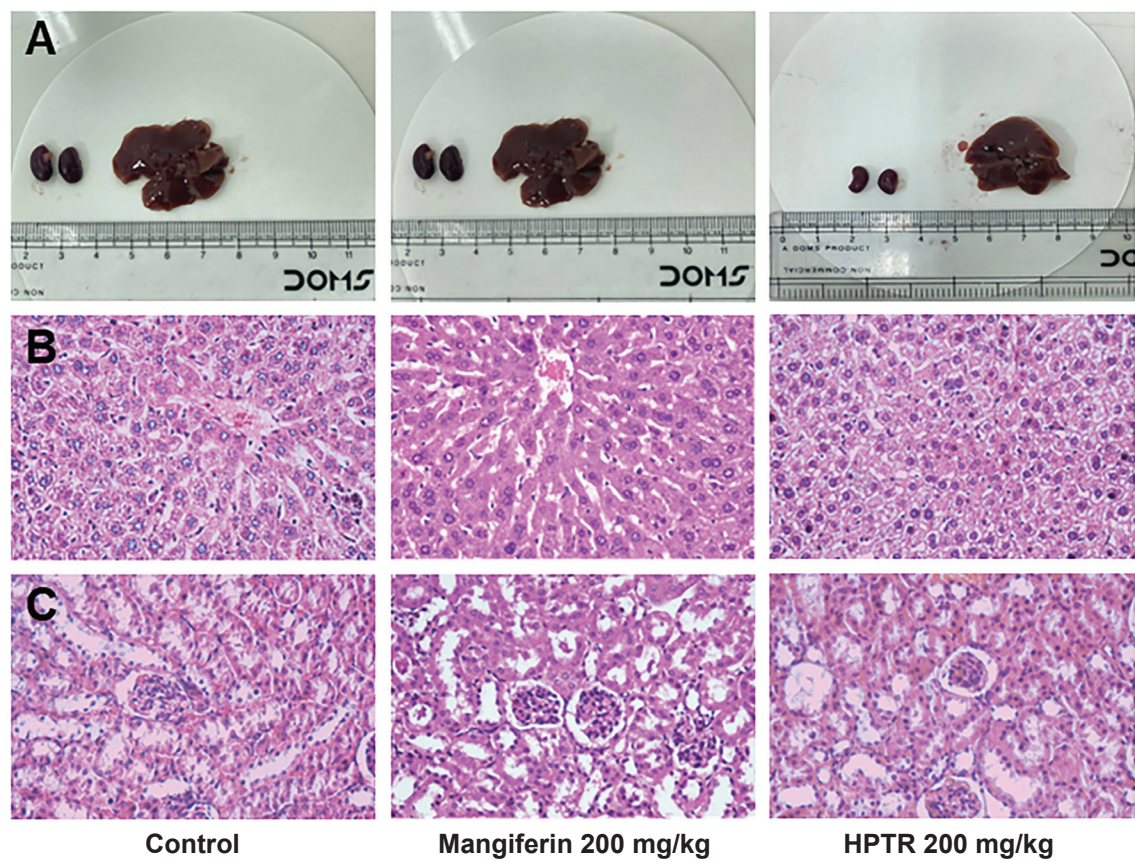


Figure 5. A Macroscopy and B microscopy of livers and C Microscopy of kidneys in sub-chronic oral toxicity.

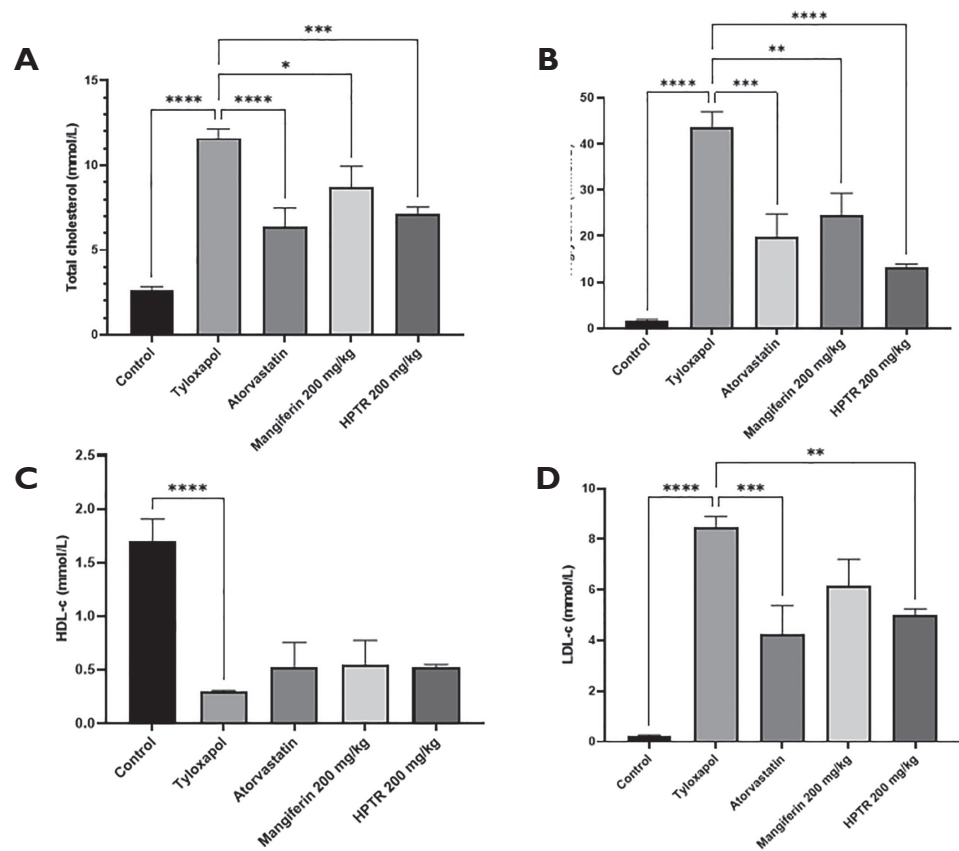


Figure 6. Effect of Mangiferin and HPTR on lipid profile in serum of mice. A. Total cholesterol; B. Triglyceride; C. HDL-c and D. LDL-c. Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. *p < 0.01 vs. control group. #p < 0.05, ##p < 0.01 vs. Tyloxapol group.

We also observed a similar trend with the level of triglyceride in the tyloxapol injected group. A significant difference in triglyceride level was documented. A Tukey post-hoc test revealed significant pairwise differences between tyloxapol injected group and control group, with an increase of 25 times. Atorvastatin, mangiferin 200 mg/kg, and HPTR 200 mg/kg showed a decrease in triglyceride level ($p < 0.05$). Although both mangiferin 200 mg/kg, and HPTR 200 mg/kg resulted in lower triglyceride levels than atorvastatin group, no significant differences were found among these groups (Fig. 6B).

Hyperlipidemia is a metabolic disorder characterized by abnormal lipid metabolism and elevated levels of serum low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), and total cholesterol (TC), as well as decreased levels of high-density lipoprotein cholesterol (HDL-c). Interestingly, while we found that HDL-c level was significantly decreased following the injection of tyloxapol ($p < 0.01$), neither atorvastatin nor mangiferin 200 mg/kg treated group showed any improvement of HDL-c level. Only HPTR 200 mg/kg showed a significant improvement in HDL-c level (Fig. 6C).

The LDL-c level showed that only mangiferin at a dose of 200 mg/kg was effective in reducing LDL-c, but this reduction was not statistically significant compared to the tyloxapol group ($p > 0.05$). HPTR 200 mg/kg and atorvastatin group also reduced LDL-c, and this reduction was significant compared to tyloxapol group ($p < 0.05$). HPTR 200 mg/kg was more effective in lowering LDL-c than mangiferin; this difference was not statistically significant ($p > 0.05$) (Fig. 6D).

Discussion

As for the classical extraction method, the results showed that ethanol 60% brought about the best extraction efficiency. With a lower ethanol concentration, mangiferin extraction was not highly effective because mangiferin is poorly soluble in water. However, when the ethanol concentration was too high, the extraction efficiency was also low because the extraction time would be long which led to solvent evaporation, hence a reduction in efficiency. The results were consistent with the research by Nguyen Duc Thanh et al. (2022) which surveyed mangiferin extraction from mango leaves collected in Son La with the optimal ethanol concentration of 60% (Thanh et al. 2022).

With a low solvent content (1/10 and 1/12), extracting mangiferin became more difficult because the amount of solvent was not enough to extract all the mangiferin in mango leaves which led to a decrease in contact with raw materials and inefficiency. However, if the amount of solvent used was too much, the amount of impurities in the extract would increase, thus wasting raw materials and fuel. In this study, the amount of mangiferin increased when the amount of solvent went up to 1/15, but at a ratio of 1/17, it decreased clearly. These results were similar to

the study by Nguyen Thi Truc Loan who extracted mangiferin from Acacia mango leaves. To be more specific, with a high solvent content, the results showed that the mangiferin content obtained using the classical extraction method was also low (Truc Loan 2018).

Temperature had an important effect on the extraction of mangiferin from Cat Chu mango leaves. This was due to the fact that at high temperature, the mass transfer occurred quickly which helped reduce viscosity and increase the solubility and diffusion of mangiferin. The extraction was conducted at normal temperature with water bath extraction at temperatures ranging from 40 °C to 60 °C (not at higher temperatures to limit the evaporation of alcohol). As a result, 60 °C was the most suitable temperature for the extraction of mangiferin using the classical extraction method. This outcome was similar to the research by Kulkarni VM et al. which surveyed extraction temperatures from 30 °C to 70 °C. The result showed that the efficiency gradually increased along with the rise in temperature from 30 °C to 60 °C and was most optimal at 60 °C (Kulkarni and Rathod 2014). Furthermore, the results were consistent with the study by K. Anbalagan et al. which indicated the increase in mangiferin content when raising extraction temperature. Moreover, the extraction efficiency increased at a temperature of 40–70 °C (Anbalagan et al. 2019) with the most optimal temperature of 70 °C. There was a slight difference between K. Anbalagan's study and this study. It was due to the difference in extraction times between the two studies. The extraction time in K. Anbalagan's study was 6 hours, while in this study the extraction was conducted in 9 hours.

The most optimal soaking time for extracting mangiferin using the classical extraction method was 9 hours. With a shorter soaking time (6 hours), the mangiferin extraction was not very effective because there was not enough time for mangiferin to dissolve and diffuse in the solvent. In this study, when the soaking time rose to 18 hours and 24 hours, the extraction efficiency did change much. This was similar to the fact that when the extraction time increased, the amount of mangiferin did not dissolve in the solvent. This result was completely consistent with the study by Pham Ngoc Khoi and Phung Thanh Son on the optimization of fish mint extraction. With a soaking time of 2 hours, the mangiferin extraction using Soxhlet method also achieved high results (Khoi and Son 2017). However, the above study also showed that when the soaking time increased further (3 hours or 4 hours), the extraction of mangiferin remained.

With the ultrasonic extraction method, ethanol 70% gave the highest efficiency and the most mangiferin content. This was the same as the result in Nguyen Thi Truc Loan's research which illustrated that with lower ethanol concentration (< 70%), mangiferin content obtained after extraction using the ultrasound-assisted extraction method was also lower (Truc Loan 2018). When the ethanol concentration was too low, the short extraction time was not enough to fully extract the active ingredient.

However, when the ethanol concentration was too high, the active ingredient content did not go up but slightly declined probably because foam would form during the ultrasonic beating process and hinder the dissolution of the extract. This was also consistent with the research by Nguyen Thi Truc Loan which indicated that high alcohol concentration (80% or 90%) caused a reduction in extraction efficiency.

Similar to the survey of the ratio of plant/solvent in the classical extraction method, 1/15 was still the most optimal condition in the ultrasonic extraction method. Regarding the ultrasound time, 20 minutes was the optimal time for the mangiferin extraction using the ultrasonic extraction method. With lower ultrasound times (< 20 minutes), mangiferin extraction was not highly effective because the time was not enough to fully extract the amount of mangiferin in mango leaves, leading to low extraction efficiency. In addition, the results were also consistent with that of the study on mangiferin in mango leaves by Zou TB et al. which showed that extraction efficiency increased when increasing ultrasound time from 5 to 20 minutes, but then decreased when the time was longer than 20 minutes (Zou et al. 2014). This might be because increasing ultrasound time could promote absorption, dissolution, and diffusion. However, the mangiferin content could decompose after a long period of exposure to ultrasound radiation, leading to a decrease in the efficiency.

The optimal amplitude for ultrasonic extraction of mangiferin was ethanol 70%. When the amplitude increased to 80%, 90%, and 100%, the mangiferin content gradually decreased. The reason might be that the higher the amplitude was, the greater the decomposition of active ingredients was. At that time, there was a heat release which resulted in the evaporation of the solvent. The research by Nuttawan Yoswathana concluded that raising ultrasound amplitudes from 25% to 75% helped enhance the efficiency of xanthone extraction from dried Mangosteen fruit peel (Yoswathana 2013). Moreover, the extraction efficiency reached the most optimal point at an amplitude of 50%.

The subchronic toxicity test was conducted at a dosage of 200 mg/kg. Observations over 28 days showed that all mice remained healthy, alert, agile, and showed normal feeding and defecation behaviors, with no deaths recorded in any group. Those data indicated that mangiferin and HPTR are safe to use for a long time.

Hematological parameters include characteristics of red blood cells (RBC), white blood cells (WBC), platelets (PLT), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). The results from Table 5 indicate that the RBC, Hb, HCT, MCV, MCH, PLT, and WBC counts for all three test groups were within normal limits and showed no significant difference ($p > 0.05$) when compared with the control group. Therefore, it can be inferred that the blood-forming function of the bone marrow is still normal, and these indices suggest that the HPTR and mangiferin

does not adversely affect health, general organ function, or specifically the blood-forming organs in the body.

In this study, the test results from Table 6 showed that at a dose of 200 mg/kg, there was an increase in AST level and a decrease in ALT compared to the control group. However, this difference was still within the normal range for mice according to Wolford et al. (1986) and was not significant when compared to the control group ($p > 0.05$) (Wolford et al. 1986).

In order to more directly assess the impact of the test sample on the liver, after the experiment was conducted, the mice were dissected to observe the liver grossly and store samples for histological staining. Gross observation (Fig. 5) showed no pathological changes in terms of size, color, or liver structure. The livers in both the test and control groups had a similar size (about 2.5 cm in diameter), were bright red in color, uniform, smooth on the surface, not porous, soft, and showed no signs of congestion or edema. Histological staining results showed that the liver samples did not differ from the control group, with uniformly sized liver cells, no necrotic inflammation foci, and the central vein not congested. The cytoplasm of the cells stained pink smoothly, and the cell nuclei were round and clear. Thus, it can be concluded that the HPTR and mangiferin did not negatively affect the liver or liver function.

The biochemical serum test results from Table 6 showed no statistical difference ($p > 0.05$) in both urea and creatinine concentrations between the test groups and the control group. In terms of histology, no gross anatomical differences were observed between the control group and the extract groups. The dissected kidneys all had a normal size (0.6–0.8 cm), were pink-red in color, and were not swollen or edematous. These results are also consistent with the kidney function test showing normal blood creatinine levels. Kidney histology as shown in Fig. 5 revealed normal glomerular morphology without cell proliferation. The renal tubular cells did not necrotize, and the stroma showed no signs of inflammation or fibrosis. There was no difference in the renal microstructure between the extract groups and the control. Thus, it can be concluded that the extract and HPTR did not negatively affect the kidneys or kidney function.

In summary, the subchronic toxicity study results in this research show that the HPTR and mangiferin at a dose of 200 mg/kg did not cause toxicity in mice after 28 days of administration. Currently, there are many studies on the toxicity of mangiferin worldwide. The study by Yalena Prado et al. (2015) assessing the semi-chronic toxicity in mice of Mangiferin extracted with methanol at 92% purity showed that at doses of 250 and 500 mg/kg, all monitored indicators including general condition, weight, hematology, liver and kidney function of mice were within normal limits (Prado et al. 2015). Despite differences in dosage used, both the above study and this study have reached the same conclusion on the safety of mango leaf medicine, providing a reliable basis for future research.

Tyloxapol (Triton WR-1339) is a surfactant that inhibits lipoprotein lipase, with the advantages of being quick,

time and cost-saving to conduct research on many groups. The model chosen in this study is the induction of endogenous lipid disorders using a dose of 400 mg/kg of tyloxapol injected intraperitoneally (Huynh et al. 2024). This dose of tyloxapol can cause a state of lipid disorder that lasts up to 24 hours in experimental animals.

Based on the lipid blood test results from our study, the tyloxapol group after 24 hours of tyloxapol injection induced a typical change in all four blood lipid parameters: triglycerides, total cholesterol, HDL-c, and LDL-c. Specifically, the tyloxapol group showed a 25.72-fold increase in triglyceride levels, a 4.45-fold increase in total cholesterol, and a 38.59-fold increase in LDL-c compared to the control group, while the HDL-c level of the tyloxapol group decreased by 5.67 times, with all differences being statistically significant ($p < 0.05$). Thus, the model induced by tyloxapol is successful and reliable. The therapeutic effect of HPTR and mangiferin after 24 hours of tyloxapol injection can be based on all four parameters: triglyceride levels, total cholesterol levels, HDL-c, and LDL-c concentrations.

When evaluating the effect of blood lipid-lowering, statins are the first choice of drugs, used alone or in combination with other drugs to achieve treatment goals or when the patient is intolerant. Atorvastatin is a popular and typical drug of the statin group, proven to treat hyperlipidemia since 1987. The mechanism of atorvastatin, and statins in general, is to inhibit cholesterol synthesis in the liver, increase the number of LDL-c receptors to help bring LDL-c into the cell, and lower triglycerides by increasing the excretion of VLDL-c. Atorvastatin was chosen as a positive control because of its advantages such as having a long half-life and not being affected by food; most importantly, it can reduce LDL-c by about 34–54% depending on the dose from 10–80 mg. This effect is second only to rosuvastatin at the maximum dose of 40 mg. Moreover, atorvastatin is quite popular due to its affordability and accessibility, and can be a preferred choice for patients with renal failure.

The lipid blood test results in the study showed that the atorvastatin control group only showed a decrease in triglycerides, total cholesterol, and LDL-c compared to the tyloxapol group. However, only the levels of triglycerides and total cholesterol were statistically significant ($p < 0.05$), while the HDL-c parameter improved but was not statistically significant ($p > 0.05$).

Both mangiferin and HPTR at a dose of 200 mg/kg showed a positive effect of enhancing blood lipid parameters. The results drew a comparison that the HPTR seemed to be more effective in decreasing total cholesterol, triglycerides, LDL-c levels and increasing HDL-c level than the mangiferin to some extent, though the differences between these two groups were not statistically significant.

When comparing the results of blood lipid regulation of the HPTR and mangiferin with some other medicinal

studies, the HPTR and mangiferin showed promising results. The study by Shiv Vardan Singh (2016) on mice with endogenous hyperlipidemia induced by tyloxapol at a dose of 400 mg/kg reported that the hydro-methanolic extract of *Flacourtia indica* at a dose of 150 mg/kg reduced total cholesterol by 17%, triglycerides by 13%, and LDL-c by 22%, and increased HDL-c by 15% compared to the tyloxapol group (Singh et al. 2016). Meanwhile, in our study, the mangiferin at the dose of 200 mg/kg achieved a 43.54% reduction in triglycerides, a 24.63% decrease in total cholesterol, an 83.33% increase in HDL-c, and a 27.56% reduction in LDL-c. Similarly, HPTR at a dose of 200 mg/kg showed a superior effect in reducing triglyceride and total cholesterol levels, respectively equivalent to 5.3 times (69.45% compared to 13%) and 2.3 times (38.33% compared to 17%) compared to the hydro-methanolic extract of *F. indica* at a dose of 150 mg/kg.

It can be seen that both atorvastatin and the high dose of 200 mg/kg did not significantly improve the HDL-c parameter, possibly because the dosage used was still not sufficient to have a significant effect in increasing HDL-c. Therefore, if we only evaluate the effect of controlling hyperlipidemia through the remaining three parameters, it is clear that HPTR at a dose of 200 mg/kg is the most promising, having a better impact in reducing triglycerides, total cholesterol, and LDL-c than the mangiferin at 200 mg/kg and equivalent to atorvastatin group. Thus, the effect of HPTR in controlling blood lipid disorders is improved compared to the mangiferin.

Our findings were consistent with the study of Sandoval-Gallegos et al., which investigated the antihyperlipidemic effect of *Mangifera indica* leaf extract containing mainly mangiferin on high cholesterol diet-fed mice. Their study showed that, at doses of 100, 200 and 400 mg/kg, *M. indica* leaf extract helped reduce total cholesterol and triglycerides levels significantly (Sandoval-Gallegos et al. 2018). Shah et al. also reported that *M. indica* leaf extract 200 mg/kg was effective in reducing LDL-c level and increasing HDL-c level as compared to the untreated group (Shah et al. 2010). Therefore, mangiferin extracted using the ultrasonic method was proved to have beneficial effect of antihyperlipidemia and it being converted into HPTR played a crucial role in enhancing this activity. The hypolipidemic activity of mangiferin is related to both lipogenesis and lipolysis processes. Mangiferin reduces enzymes such as acetyl-CoA carboxylase (Acc), diglyceride acyltransferase (Dgat), stearoyl-CoA desaturase (Scd) and so on, which participate in the lipogenesis process. In terms of lipolysis, mangiferin stimulates this process via increasing the mRNA of LPL, which catalyses the hydrolysis of triglycerides to free fatty acids, and inducing CD36, which helps translocate the free fatty acids from the circulation into cells (Fomenko and Chi 2016).

From these results, it can be concluded that the HPTR and mangiferin have the ability to control hyperlipidemia in mice caused by tyloxapol, with var-

ious mechanisms for reducing blood lipids such as the ability to regulate the reduction of proteins controlling the De novo lipogenesis (DNL) process as studied by Ji-hyeon Lim and colleagues (2014) (Lim et al. 2014). The effect of controlling hyperlipidemia by the HPTR and mangiferin equivalent to atorvastatin at the dose of 200 mg/kg had a 27.56% reduction in LDL-c. In addition, the effect of HPTR in controlling blood lipid disorders is improved compared to the mangiferin but the difference was not statistically significant.

Conclusion

In conclusion, our study successfully extracted mangiferin using the ultrasonic method and prepared a solid dispersion (HPTR) to increase the solubility of mangiferin, yet maintained its effect to control hyperlipidemia in mice caused by tyloxapol. HPTR seemed to be more effective in decreasing total cholesterol, triglycerides, LDL-c levels and increasing HDL-c level than mangiferin to some extent, though the differences between these two groups were not statistically significant.

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CRediT authorship contribution statement

D.T.M.H: First author: Methodology, Conceptualization, Writing – original draft, Project administration, Resources, Writing – Review & editing, Supervision; L.M.L., L.T.N., T.H.N.N., M.H.N., K.T.V.N., K.Q.T., T.L.Q.T.: Methodology, Conceptualization, Writing – original draft; M.N.T.L: Methodology, Conceptualization, Resources; H.N.M: Corresponding author: Writing – original draft, Data curation, Resources, Writing – Review & editing.

Declaration of competing interest

The authors have declared that no competing interests exist.

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