Journal of Applied Pharmaceutical Science Vol. 13(02), pp 021-028, Febuary, 2023
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2023.130203
ISSN 2231-3354

Long-term oral administration of *Paederia foetida* decreases cytochrome P450 mRNA expression: The predictive approaches in a rat model

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ARTICLE INFO

Received on: 14/07/2022
Accepted on: 14/11/2022
Available Online: 05/02/2023

Key words:
Cytochrome p450 expression, Cyp3a1, Cyp2d1, Cyp2c6, *Paederia foetida*, liver toxicity, kidney toxicity.

ABSTRACT

Paederia foetida (Linn.) is one of the medicinal plants containing various bioactive compounds (e.g., beta-sitosterol and ursolic acid), which has been widely used in medicine and cuisine among different communities. It exhibits several pharmacological properties, including anti-inflammatory, antioxidant, antidiabetic, and anticancer. However, its toxicity on the liver and kidney, as well as its effect on hepatic drug-metabolizing enzymes, lacks documentation. Therefore, the toxic effects of *P. foetida* on the liver and kidney and metabolizing enzymes were explored. In a recent study, male Wistar rats were grouped into five groups and gavaged daily with 0–1,000 mg/kg BW of a *P. foetida* extract (PFE) for 8 weeks. After euthanasia, liver, kidney, and blood samples were collected and subjected to histological and biochemical analysis. The important cytochrome P450 mainly expressed in the rat liver, including *Cyp3a1, Cyp2d1,* and *Cyp2c6*, was investigated. There were no changes in body and organ weight between groups. However, the liver weight of rats treated with 1,000 mg/kg BW PFE was increased when compared to the control group. The liver and kidney histopathology were observed with no difference between groups. The markers of liver damage (alanine aminotransferase and aspartate aminotransferase) and kidney damage (blood urea nitrogen and creatinine) between all groups showed no change. *Cyp3a1*, *Cyp2d1*, and *Cyp2c6* mRNA expression levels among PFE-treated groups were decreased in a dose-dependent manner, suggesting that subchronic exposure to this extract significantly reduces *Cyp3a1*, *Cyp2d1*, and *Cyp2c6* expressions in rat livers without toxicity or change in histology and biochemical data. However, its concomitant use with prescription drugs needs further investigation.

INTRODUCTION

Paederia foetida (Linn.) (Rubiaceae family) has been used in Thai cuisine and Thai traditional medicine for diseases, including rheumatoid arthritis (Dixit, 2013; Kumar *et al*., 2015), diarrhea (Afroz *et al*., 2006), asthma (Macwan, 2010), and relieving diabetes (Kumar, 2014), in the southern region including Thailand. Phytochemical analysis revealed that *P. foetida* is rich in lupeol, ursolic acid, and beta-sitosterol (Dwivedi *et al*., 2018), which are shown to have their biological activities, including anti-inflammatory (Das *et al*., 2012), antioxidant (Upadhyaya, 2013), antidiabetic (Morshed *et al*., 2012), and anticancer (Reddy *et al*., 2011) activities. Although the use of this plant is widespread, information about its toxicity-related issues is still limited (Reddy *et al*., 2011). Moreover, the long-term use of herbal remedies has also been associated with liver toxicity (Teschke *et al*., 2013), adverse effects on kidney function (Amadi and Orisakwe, 2018), and herb-drug interaction (Hu *et al*., 2005). To better understand *P. foetida* use and toxicity-related issues, it is important to promote awareness of possible health complications and promote the safety

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of long-term effects of *P. foetida* accumulation in rat organs and toxicity associated with herbs (Turkmenoglu *et al*., 2016).

After xenobiotics enter the human body, they undergo further metabolism (biotransformation), mainly in the liver. In the liver, xenobiotic sensors, e.g., pregnane X receptor (PXR) and constitutive androstane receptor (CAR), coordinate the transcriptional control of the P450-metabolizing enzyme, which catalyze rate-limiting steps in xenobiotic clearance or biotransformation (Chai *et al*., 2016; Chang *et al*., 2017). Moreover, more than 75% of pharmaceutical drugs undergo first-pass metabolism in the liver by cytochrome p450 enzymes (Wienkers and Heath, 2005). Therefore, the biotransformation of xenobiotics was expected to alter the function of drug-metabolizing P450 enzymes, leading to therapeutic failure or the activation of toxicity mechanisms. In previous reports, herbal plants were shown to cause *in vitro* CYP inhibition and upregulation and downregulation of CYP mRNA expression, resulting in toxic effects (Gravel *et al*., 2018). However, the impact of the *P. foetida* ethanol extract on *in vivo* mRNA expression was less well documented.

There is no published report on the effect of the *P. foetida* ethanol extract on the subchronic toxicity and CYP mRNA expression in animal models. Therefore, this study aimed to investigate the effects of *P. foetida* on i) subchronic toxicity concerning damage to the liver and kidney function and ii) the alteration of the mRNA expression level of hepatic phase I drug-metabolizing enzymes in rats. To predict and avoid herb-drug interaction, the predominant forms of cytochrome p450 in rat livers, including *Cyp3a1*, *Cyp2d1*, and *Cyp2c6*, have been considered in this study (Martignoni *et al*., 2006).

MATERIALS AND METHODS

Plant materials

Paederia foetida leaves (voucher sample: Ref. HA1601061505) were obtained from Pak Phanang, Nakhon Si Thammarat, Thailand (8°17′54.5″N 100°09′13.1″E). *Paederia foetida* leaves were cut into small pieces and subjected to a hot air oven for 48 hours at a temperature of 60°C. *Paederia foetida* leaves powder was soaked in 95% ethanol for 3 days (three times) at the ratio of a 100 g sample: 1 L ethanol. After ethanol evaporation, a crude semisolid residue was obtained with a percentage yield value of 20 /100 g (dried *P. foetida* leaf powder).

High Performance Liquid Chromatography (HPLC) and mass analysis

100 µg of the *P. foetida* leaves ethanol extract was subjected to HPLC.. The separation was performed using a reverse phase C18 column as the stationary phase, whereas the mobile phase contained water (A) and acetonitrile (B). The peak chromatogram of the *P. foetida* leaves ethanol extract was eluted by the condition which was set as the following: 0–30 minutes, 25%–100% B; 30–45 minutes, 100% B; 45–50 minutes, 100%–25% B; 50–60 minutes, 25% B. The major peak eluted at 12.48 minutes was collected and subjected to a mass analyzer (directed methods). Mass per charge ratio was employed by electrospray ionization (ESI) (Bruker Daltonics GmbH, Bremen, Germany). The mass spectrometry (MS) spectra were acquired in positive modes, and m/z were scanned in the range of 100–1,000 and acquired by HyStar (V3.2). Parameters for MS were as follows: nebulizer gas (nitrogen): 9 l/minute, spray voltage: 4,000 V, ESI soured temperature: 365°C, and collision gas (helium) pressure: 9.6×10^{-6} Torr. Bruker Daltonics Data analysis version 3.1 was used for ion chromatogram extraction.

Animals

The experimental animals (7-week-old male Wistar rats weighing 170–200 g) in this study were obtained from the National Laboratory Animal Center, Nakhon Pathom, Thailand. After arrival at the Walailak Animal Center, the animals were acclimatized before the experiment for 5 days and housed under controlled conditions of 22°C ± 2°C, 55% ± 10% humidity, and a 12:12 hours light: dark cycle with food and water *ad libitum*. The research protocol for the animal study was approved by the Institutional Animal Care and Use Committee, Walailak University (WU-AICUC-63008).

Experimental design

After the acclimation period, rats were randomly assigned into five groups ($n = 6$). Each group was treated daily (p.o.) with either 0.25% CMC for the control group or 50, 100, 500, and 1,000 mg/kg PFE for the treated groups. Moreover, the rats were checked once a day for signs or symptoms of toxicity including illness, injury, and abnormal behavior. After 8 weeks, a gross necropsy and complete gross postmortem examinations were performed after all animals were euthanized. Organs, including the brain, spleen, liver, and kidneys, were collected and weighed to further calculate absolute and relative organ weights (organ weight (g)/body weight (g) × 100). Blood samples were collected and subjected to blood biochemistry parameter analysis. The liver and kidneys were fixed in 10% neutral buffered formaldehyde for further histological analysis, and a part of the tissues was stored at −80°C after being snap-frozen in liquid nitrogen.

Hematology and biochemical studies

At the end of the experiment, rats were intraperitoneally injected with Zoletil 100 and xylazine at the ratio of 25:5 mg/kg BW (Machado *et al*., 2009). After rats were deeply anesthetized, blood samples were collected by a cardiac puncture and kept in ethylenediamine tetraacetic acid tubes. Complete blood count, including i) red blood cell (RBC) count, ii) white blood cell (WBC) count, iii) hemoglobin (Hb) concentration, iv) % hematocrit (Hct), as well as RBC indices including v) mean corpuscular volume (MCV), vi) mean corpuscular hemoglobin (MCH), and vii) mean corpuscular hemoglobin concentration (MCHC), was determined by the Auto Hematology Analyzer (Aspen Diagnostics, India). For other biochemical studies, serum was collected by centrifugation at 3,000 rpm for 30 minutes. After that, the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine were determined by using an automated analyzer (Mindray BS-400 Chemistry Analyzer, Aspen Diagnostics, India).

Histopathological study

After blood collection, the liver and kidneys were excised and fixed in 10% buffered formalin. Liver and kidney

tissue were embedded in paraffin wax and were cut at 5 µm thick sections. The histology of the liver and kidneys was determined using staining with hematoxylin and eosin dye. The histological slides and representative photomicrographs were examined and captured under a light microscope.

RNA isolation and quantitative real-time polymerase chain reaction (PCR)

The RNeasy Mini Kit (QIAGEN, Germantown, MD) was used to extract mRNA, and 1 µg of total mRNA was then converted to cDNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The specific primers for *Cyp3a23/3a1*, *Cyp2d1*, and *Cyp2c6* were purchased from Applied Biosystems (Foster City, CA): *Cyp3a23/3a1* (Rn01640761_gH), *Cyp2d1* (Rn01775090_mH), *Cyp2c6* (Rn02329961_mH), and *Gapdh* (Rn01775763_g1). Gene expression of target genes was amplified by real-time PCR reactions in triplicate in an ABI 7500 Sequence Detection System (Applied Biosystems). The expression of *Cyp3a23/3a1*, *Cyp2d1*, and *Cyp2c6* genes was normalized to *Gapdh,* and quantification of relative expression was determined by Pfaffl's method (Pfaffl, 2001).

Statistical analysis

Data were analyzed using the Prism 9 software (GraphPad, La Jolla, CA) and presented as means ± SD. Mean differences between groups were performed using a two-tailed unpaired Student's *t*-test or one-way analysis of variance. Significant differences were considered when the *p*-value was less than 0.05.

RESULTS

HPLC analysis and PFE

We preliminarily determined the constituents of the *P. foetida* ethanol extract (PFE); 100 µg of the crude ethanol extract of the *P. foetida* ethanol extract was subjected to the HPLC and mass analyzer. The major peak of the *P. foetida* ethanol extract under our condition was eluted at 12.48 minutes (Fig. 1A). According to the mass analyzer, 2 ion mass $[M-H]^+ = 413$ (Fig. 1B) and $[M+H]^+ = 457$ (Fig. 1C) were observed and identified as beta-sitosterol (Rozenberg *et al*., 2003) and ursolic acid (Novotny *et al*., 2003), which are consistent with the previous report, respectively.

Changes in body weight and organ weight

To determine the toxic effect of *P. foetida*, rats were treated with different doses of PFE (0–1,000 mg/kg). The change in body and organ weight was firstly determined as an indicator of toxic effects (Lazic *et al*., 2020). The results showed that the change in body weight among the treated group was not observed up to 1,000 mg/kg/day compared to the control group (Table 1), and there was no sign of toxicity and morbidity. Moreover, organ and relative organ weights (Table 2), including brain, spleen, and kidney weight, were not significantly different between the treatment and control groups. However, the liver weight and relative liver weight of the 1,000 mg/kg PFE-treated group were slightly increased compared to the control groups. The results indicate that subchronic effects of PFE have no potential toxic effects on the body and organ weight up to 1,000 mg/kg body weight.

Hematology parameters

We next determined the effect of the *P. foetida* ethanol extract on hematology parameters which is a sensitive index for vital toxicity (s). In Table 3, there was no significant change in hematological values, including RBC count, Hb, Hct, platelet, and WBC count (e.g., neutrophils, lymphocytes), in the rats treated with PFE compared with the control group. This result suggests that the *P. foetida* constituents in the ethanol extract had a low toxic effect on the hematologic system, which was basically in the normal reference range.

Effect of PFE on liver and kidney functions

To investigate the effect of PFE on the liver and kidneys, which play important roles in xenobiotic metabolism and clearance (Woldemeskel, 2017), histology and biochemistry parameters were determined. As shown in Figures 2 and 3, no lesions were found upon macroscopic and microscopic examinations in either the liver or kidney tissue of any of the rats treated with *P. foetida,* as the cellular integrity and the cellular architecture were intact in both tissues compared to controls. Aside from the gross appearance and histology of the liver and kidneys, the biomarkers reflecting liver (serum ALT and AST) and kidney (serum BUN and creatinine) damage were then determined. The results revealed that all biochemical parameters for liver and kidney damage were within physiological ranges and were not significantly different in the PFE-treated group and control group, suggesting that subchronic administration of PFE might have no nephrotoxic and hepatotoxic effects.

In vivo effect of PFE on major cytochrome P450 mRNA expression

Paederia foetida was previously reported to inhibit *in vitro* cytochrome P450 activity (Dubey *et al*., 2017). However, altered expression of major cytochrome P450 enzymes *in vivo* is still unreported. Therefore, mRNA expressions of *Cyp3a1*, *Cyp2c6*, and *Cyp2d1* in the rat liver of each treated group were determined using target-specific primers in real-time PCR. As shown in Figure 4, at 100 mg/kg of treatment, mRNA expression levels of *Cyp3a1* and *Cyp2d1* were 2-fold lower than that observed in the control group. However, mRNA expression levels of *Cyp2c6* showed a significant decrease from that observed in the control group at 500 and 1,000 mg/kg. Therefore, the administration of *P. foetida* decreases mRNA expression levels of *Cyp3a1*, *Cyp2d1*, and *Cyp2c6* in a dose-dependent manner, suggesting that *P. foetida* may interfere with xenosensor and cytochrome P450 activity.

DISCUSSION

The use of herbal supplements, both individually and in combination with prescription drugs, is continuing to increase among the global population. The naturally occurring phytochemicals in plants are abundant sources of therapeutic agents for medicinal purposes in developing countries. Although plants show the benefit needed in health maintenance, the available evidence revealed that people are frequently exposed

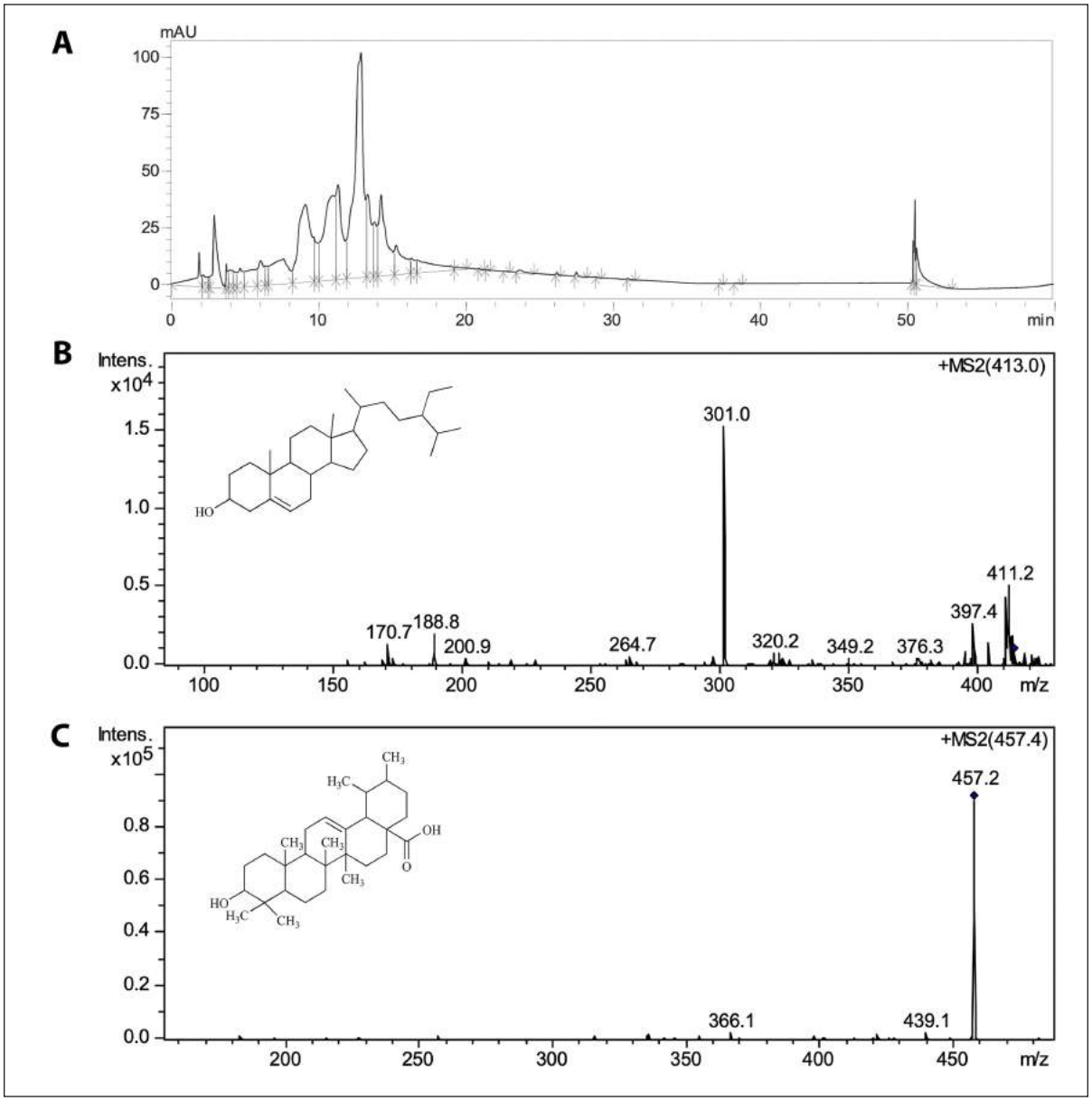

Figure 1. The major compounds in PFE are beta-sitosterol and ursolic acid. (A) HPLC chromatogram of 1 mg/ml (100 µg) chemical constituents of *P. foetida* ethanol extract. The major peak was eluted at 12.48 minutes and subjected to a mass analyzer. Two major mass ion (parent ion) chromatogram of 413 $[M-H]^+$ [daughter ion chromatogram (MS/MS) of 397 $[M+H-H2O]^+$] and mass ion (parent ion) chromatogram of 457 $[M+H]^+$ [daughter ion chromatogram (MS/MS) of 439 $[M+H-OH]^+$] were observed and identified as (B) Beta-sitosterol, and (C) Ursolic acid, respectively.

Table 1. Effects of the PFE on body weight gain in the rats with lead acetate toxicity.

Parameter	Vehicle control	*Paederia foetida* ethanol extract (mg/kg/day)			
		50	100	500	1,000
Initial BW (g)	226.2 ± 2.2	228.4 ± 3.5	228.4 ± 3.1	226.6 ± 3.6	225.0 ± 2.9
Final BW (g)	449.2 ± 5.1	457.1 ± 19.4	452.4 ± 9.2	456.9 ± 4.6	454.5 ± 12.5
BW gain					
g	223.0 ± 5.5	228.7 ± 18.8	224.0 ± 9.1	230.3 ± 6.2	229.5 ± 11.6
% relative	100.0 ± 2.5	102.6 ± 8.4	100.5 ± 4.1	103.3 ± 2.8	102.9 ± 5.2

There is no difference between groups ($p > 0.05$).

Table 2. Effects of the PFE on organ weight ratio (% body weight, BW).

Parameter	Vehicle control	*Paederia foetida* ethanol extract (mg/kg/day)			
		50	100	500	1,000
Brain					
Organ weight (g)	1.99 ± 0.03	1.98 ± 0.04	2.02 ± 0.06	1.99 ± 0.02	2.00 ± 0.02
Relative organ weight (% BW)	0.44 ± 0.01	0.44 ± 0.02	0.45 ± 0.01	0.44 ± 0.01	0.45 ± 0.01
Spleen					
Organ weight (g)	0.81 ± 0.03	0.81 ± 0.01	0.84 ± 0.03	0.85 ± 0.03	0.88 ± 0.02
Relative organ weight (% BW)	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Liver					
Organ weight (g)	14.73 ± 0.33	14.87 ± 0.66	15.17 ± 0.47	15.78 ± 0.66	16.26 ± 0.40*
Relative organ weight (% BW)	3.28 ± 0.09	3.27 ± 0.12	3.33 ± 0.09	3.46 ± 0.16	3.59 ± 0.11*
Kidney					
Organ weight (g)	3.30 ± 0.11	3.38 ± 0.11	3.52 ± 0.10	3.52 ± 0.14	3.43 ± 0.24
Relative organ weight (% BW)	0.74 ± 0.03	0.74 ± 0.02	0.78 ± 0.03	0.78 ± 0.03	0.76 ± 0.06

*$p < 0.05$, compared with control ($n = 6$/group).

Table 3. Effect of subchronic exposure to PFE on hematological values.

Parameter	Vehicle control	*Paederia foetida* ethanol extract (mg/kg/day)			
		50	100	500	1,000
RBC (× 10^6/µl)	6.55 ± 0.15	6.21 ± 0.12	6.20 ± 0.12	6.31 ± 0.17	6.43 ± 0.12
Hemoglobin (g/dl)	14.03 ± 0.34	14.02 ± 0.29	13.98 ± 0.32	13.87 ± 0.20	13.93 ± 0.27
Hct (%)	34.50 ± 0.43	34.29 ± 0.42	34.33 ± 0.67	34.50± 0.85	35.67 ± 0.67
MCV (fl)	53.71 ± 1.11	53.86 ±0.83	55.50 ± 0.81	54.83 ± 0.79	55.83 ± 0.48
MCH (pg)	21.50 ± 0.50	21.57 ± 0.30	21.83 ± 0.48	22.00 ±0.52	21.33 ± 0.33
MCHC (g/dl)	41.25 ± 0.85	41.43 ±0.43	39.33 ± 0.71	40.33 ±0.67	39.67 ± 0.80
RDW (%)	13.75 ± 0.25	13.43 ± 0.20	13.88 ± 0.23	14.00 ± 0.17	14.43 ± 0.30
WBC (× 10^3/µl)	5.10 ± 0.40	5.30 ± 0.025	4.89 ± 0.45	4.45 ± 0.38	4.41 ± 0.36
Neutrophils (%)	7.33 ± 1.41	8.71 ± 0.84	8.17 ± 1.35	8.17 ± 1.47	10.00 ± 1.46
Lymphocytes (%)	88.00 ± 1.23	86.71 ± 0.81	86.67 ± 1.54	87.83 ± 1.45	85.50 ± 1.12
Platelet (× 10^3/µl)	739.2 ± 36.90	742.3 ± 25.24	756.7 ± 28.54	706.8 ± 21.34	735.0 ± 28.01

There is no difference between groups ($p > 0.05$).

to various forms of toxic plants. To explore the safety use of *P. foetida*, Wistar rats were given daily oral administration with PFE (0, 50, 100, 500, and 1,000 mg/kg BW) for 8 weeks. The changes in body weight, organ weights, relative organ weight, all hematological and biochemical parameters, and liver and kidney histology were investigated. There was also no adverse change in the kidneys of the PFE-treated group compared to the control group.

Loss of body and organ weight of more than 10% is used as an indicator of adverse side effects of toxic agent exposure (Woldemeskel, 2017). Surprisingly, 8 weeks of PFE treatment showed no body weight change between the groups, and there was no sign of toxicity and morbidity. However, we found that the mean liver weight and relative liver weight were slightly increased at 1,000 mg/kg BW of the PFE-treated group, while the abnormalities (e.g., histology, biochemistry parameters for liver damage) were not observed (Fig. 2). This result is in line with our liver function biomarker results including ALT and AST, which indicate that the liver increased in size without toxicity (Olayode *et al*., 2020). The increased liver weight could be due to a functional adaptation of the liver for maintaining the homeostasis toward xenobiotics by increasing the hepatocellular protein involved in hepatocellular drug metabolism such as drug transporters, thus enhancing the rate of metabolism and excretion of the xenobiotics (Xu *et al*., 2005). In addition, most drugs or xenobiotics and their metabolites are excreted by the kidneys. Increased levels of BUN and creatinine are the hallmarks of renal failure. However, the BUN and creatinine levels in this study showed no significant changes. There was also no adverse change in the kidneys of the PFE-treated group compared to the control group. Our results were aligned with those from previous reports that demonstrated the hepato- and nephroprotection of *P. foetida* in rats, including the following: i) the administration of 500 mg/kg (p.o.) *P. foetida* methanal extract for 28 days protects the rat's

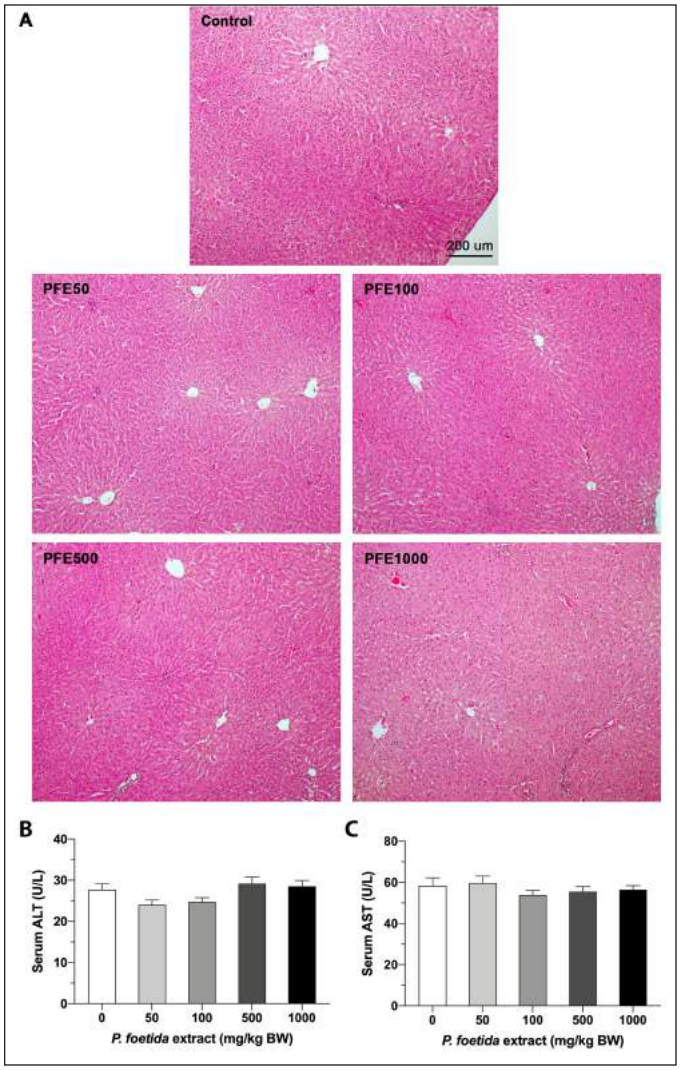

Figure 2. PFE has no hepatotoxic effects. (A) Representative photomicrographs of livers stained with hematoxylin and eosin from control and PFE-treated rats (at doses of 50, 100, 500, and 1,000 mg/kg BW). Scale bars, 200 µm. No changes were observed in biochemical parameters including. (B) Serum ALT. (C) AST levels in rats treated with PFE (n = 6/group).

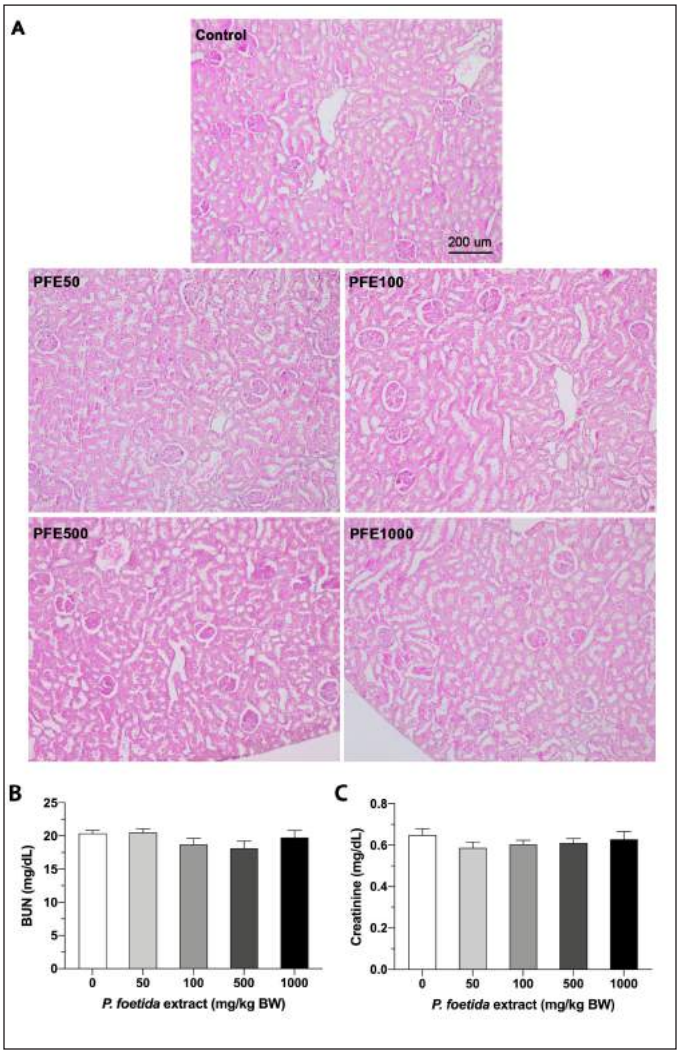

Figure 3. PFE has no nephrotoxicity in rats. (A) Representative photomicrographs of kidneys stained with hematoxylin and eosin from control and PFE-treated rats (at doses of 50, 100, 500, and 1,000 mg/kg BW). Scale bars, 200 µm. No changes were observed in biochemical parameters including. (B) Serum BUN. (C) Serum creatinine levels in rats treated with PFE (n = 6/group).

kidneys from alloxan-induced diabetic renal injury (Borgohain *et al*., 2017); ii) 200 mg/kg BW *P. foetida* ethanal extract has been shown to have a protective effect against paracetamol-induced acute liver injury (Roy *et al*., 2017); and iii) preadministration of 400–500 mg/kg BW *P. foetida* ethanol extract for 21 days protects the rat liver from CCl_4-induced liver injury (Kumar, 2014; Uddin *et al*., 2011). Several reports, including those from our study, suggest that 100–1,000 mg/kg BW of *P. foetida* intake might be a safe *in vivo* model. The concentration of 1,000 mg/kg BW of PFE in rats can be calculated to estimate the human equivalent dose (HED) by using HED (mg/kg) = animal dose (mg/kg) × km ratio, in which the km ratio is obtained from dividing 6 (animal km factor) by 37 (human km factor), which is 162 mg/kg BW. Our calculation suggests that this dose could be used in humans without any adverse effects because of its nontoxicity to the liver and kidney (Nair and Jacob, 2016).

Herein, we reported that the first-time oral administration of *P. foetida* decreased the mRNA expression levels of *Cyp3a1*, *Cyp2d1*, and *Cyp2c6* which play an important role in phase I metabolism in liver tissues of Wistar rats that received different concentrations of PFEs in a dose-dependent manner (Fig. 4). This may be due to the ability of the naturally occurring compounds in *P. foetida* to decrease the basal transcription of *Cyp3a1*, *Cyp2d1*, and *Cyp2c6* mRNA. In general, mRNA expression levels of CYP-metabolizing enzymes are regulated by xenobiotic sensors acting as nuclear transcription factors (e.g., PXR and CAR). Upon binding to xenobiotic responsive elements, the *CYP* genes turn on, and proteins are synthesized, leading to increased first-pass metabolism of xenobiotics followed by elimination from the body. We suppose that the major compounds found in the *P. foetida* extract (PFE) (beta-sitosterol and ursolic acid [a pentacyclic terpenoid, (Fig. 1)] (Dwivedi *et al*., 2018) may antagonistically

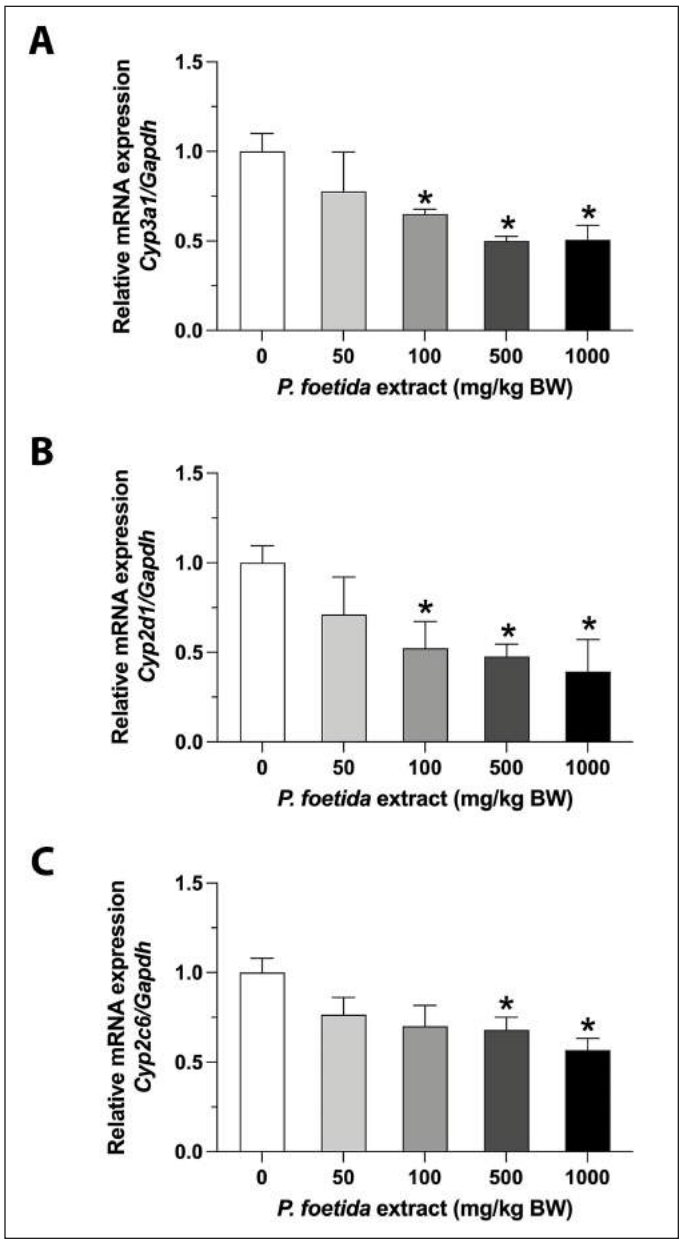

Figure 4. PFE inhibits (A) *Cyp3a1*, (B) *Cyp2d1*, and (C) *Cyp2c6* mRNA expression in livers of PFE-treated rats. *$p < 0.05$, compared with control ($n = 6$/group).

bind to the PXR and CAR nuclear receptors and decrease the mRNA expression levels of *Cyp3a1*, *Cyp2d1*, and *Cyp2c6*. This is consistent with the findings of Chang *et al*. (2017), who found that ursolic acid (pentacyclic triterpene) had an antagonistic effect against PXR and CAR activity which is considered an ortholog of PXR and CAR in rats (Jones *et al*., 2000), resulting in a decrease of both mRNA and protein expressions of CYP3A4 and CYP2B6 (Chang *et al*., 2017; Picking *et al*., 2018). According to the previous report, downregulation of *Cyp3a1*, *Cyp2d1*, and *Cyp2c6* mRNA correlated with decreasing in Cyp3a1, Cyp2d1, and Cyp2c6 activity (Zhou *et al*., 2019), suggesting that decreasing in *Cyp3a*1, *Cyp2d1*, and *Cyp2c6* mRNA might cause drug-induced toxicity by the accumulation of drugs that are metabolized by Cyp3a1, Cyp2d1, and Cyp2c6 enzymes. The antagonistic effects of *P. foetida* and pentacyclic triterpenes (e.g., beta-sitosterol, lupeol, epifriedelinol, and ursolic acid) on PXR and CAR need to be further elucidated. Moreover, rat Cyp3a1, Cyp2d1, and Cyp2c6 isozymes correspond to the human CYP3A4, CYP2D6, and CYP2C9, respectively, and they are known to be involved in the most important phase I metabolism of most drugs. Therefore, the antagonistic effect of *P. foetida* on phase I metabolizing enzymes may increase the efficacy of anticancer drugs by reducing drug clearance through the downregulation of PXR and CAR (Xing *et al*., 2020). However, high intake of *P. foetida* might harm human health and need to be determined further.

CONCLUSION

Our study revealed that PFE has no subchronic toxic effects on the kidneys and liver in an animal model. However, long-term intake of *P. foetida* needs caution as it could lead to herb-drug interactions since *P. foetida* exhibits downregulation of mRNA expression levels and may alter the functions of the key CYP isozymes found in the rat's liver, including Cyp3a1, Cyp2c6, and Cyp2d1. This could lead to a reduction in CYP activity and may result in the accumulation of xenobiotics in the body. Nevertheless, several important aspects, such as the actual concentration of ursolic acid in *P. foetida*, coadministration of herb and drug, mechanism-based enzyme inhibition, and underlying mechanisms concerning upstream nuclear receptor and downstream protein levels, need to be further elucidated for a better understanding of the cytochrome P450 expression and the complete safety profile of *P. foetida*.

CONFLICT OF INTEREST

The authors report no financial or other conflicts of interest in this work.

FUNDING

This work was supported by Walailak University, Thailand (Grant No. WU-IRG-63-024).

ETHICAL APPROVAL

The research protocol for the animal study was approved by the Institutional Animal Care and Use Committee, Walailak University (WU-AICUC-63008).

AUTHORS' CONTRIBUTION

PP, SK, SY, and TK performed the experiments. PP and TK analyzed the data and prepared the figures. PP, SY, and TK interpreted the results of the experiments. PP and TK drafted the manuscript. PP, SY, and TK edited, revised, and approved the final version of the manuscript. PP, SK, SY, and TK conceived and designed the research.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

PUBLISHER'S NOTE

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REFERENCES

Afroz S, Alamgir M, Khan MT, Jabbar S, Nahar N, Choudhuri MS. Antidiarrhoeal activity of the ethanol extract of *Paederia foetida* Linn. (Rubiaceae). J Ethnopharmacol, 2006; 105(1-2):125–30.

Amadi CN, Orisakwe OE. Herb-induced liver injuries in developing nations: an update. Toxics, 2018; 6(2):24.

Borgohain MP, Chowdhury L, Ahmed S, Bolshette N, Devasani K, Das TJ, Mohapatra A, Lahkar M. Renoprotective and antioxidative effects of methanolic Paederia foetida leaf extract on experimental diabetic nephropathy in rats. J Ethnopharmacol, 2017; 198:451–9.

Chai SC, Cherian MT, Wang YM, Chen T. Small-molecule modulators of PXR and CAR. Biochim Biophys Acta, 2016; 1859(9): 1141–54.

Chang HY, Chen CJ, Ma WC, Cheng, WK, Lin YN, Lee YR, Chen JJ, Lim YP. Modulation of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) activation by ursolic acid (UA) attenuates rifampin-isoniazid cytotoxicity. Phytomedicine, 2017; 36:37–49.

Das S, Bordoloi P, Saikia P, Kanodia L. The analgesic and acute anti-inflammatory effect of the ethanolic extract of the leaves of *Paederia foetida* (EEPF) on experimental animal models. Bangladesh J Med Sci, 2012; 11(3):206–11.

Dixit V. *Paederia foetida* Linn: phytochemistry, pharmacological and traditional uses. Int J Pharma Sci Res, 2013; 4(12):4525–30; doi:10.13040/IJPSR.0975-8232

Dubey S, Mitra K, De BK, Mondal A, Bishayee A. Effects of *Paederia foetida* and its bioactive phytochemical constituent lupeol on hepatic phase I drug metabolism. Nat Prod Commun, 2017; 12(9):1934578X1701200906.

Dwivedi J, Gupta A, Verma S, Dwivedi M, Paliwal S, Rawat AKS. Validated high-performance thin-layer chromatographic analysis of ursolic acid and β-sitosterol in the methanolic fraction of Paederia foetida L. leaves. JPC, 2018; 31(5):377–81.

Gravel S, Chiasson JL, Dallaire S, Turgeon J, Michaud V. Evaluating the impact of type 2 diabetes mellitus on CYP450 metabolic activities: protocol for a case–control pharmacokinetic study. BMJ Open, 2018; 8(2):e020922.

Hu Z, Yang X, Ho PCL, Chan SY, Heng PWS, Chan E, Duan W, Koh HL, Zhou S. Herb-drug interactions. Drugs, 2005; 65(9):1239–82.

Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NCO, LeCluyse EL, Lambert MH, Willson TM, Kliewer SA, Moore JT. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. Mol Endocrinol, 2000; 14(1):27–39.

Kumar N. Hepatoprotective activity of leaf extract of *Paederia foetida* in experimental liver cirrhosis. Inventi Rapid Ethnopharmacol, 2014; 2:1–6.

Kumar V, Al-Abbasi FA, Ahmed D, Verma A, Mujeeb M, Anwar F. *Paederia foetida* Linn. inhibits adjuvant induced arthritis by suppression of PGE(2) and COX-2 expression via nuclear factor-κB. Food Funct, 2015; 6(5):1652–66.

Lazic SE, Semenova E, Williams DP. Determining organ weight toxicity with Bayesian causal models: improving on the analysis of relative organ weights. Sci Rep, 2020; 10(1):6625.

Machado EF, Normand AC, Nunes LA, Brenzikofer R, Macedo DV. Effects of different general anesthetics on serum hemolysis and hepatic and muscular glycogenolysis in rats. Braz J Med Biol Res, 2009; 42(11):1035–8.

Macwan C. *Paederia foetida* Linn. As a potential medicinal plant: a review. J Pharm Res, 2010; 3:3135–7.

Martignoni M, Groothuis GM, Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol, 2006; 2(6):875–94.

Morshed H, Islam M, Parvin S, Ahmed MU, Islam MS, Mostofa A, Sayeed MSB. Antimicrobial and cytotoxic activity of the methanol extract of *Paederia foetida* Linn.(Rubiaceae). J Appl Pharm Sci, 2012; 2(1):77.

Nair AB, Jacob SA. Simple practice guide for dose conversion between animals and human. J Basic Clin Pharm, 2016; 7(2):27–31.

Novotny L, Abdel-Hamid ME, Hamza H, Masterova I, Grancai D. Development of LC–MS method for determination of ursolic acid: application to the analysis of ursolic acid in Staphylea holocarpa Hemsl. J Pharm Biomed Anal, 2003; 31(5):961–8.

Olayode OA, Daniyan MO, Olayiwola G. Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of *Stachytarpheta cayennensis* in rats. J Trad Complement Med, 2020; 10(6):544–54.

Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res, 2001; 29(9):e45.

Picking D, Chambers B, Barker J, Shah I, Porter R, Naughton DP, Delgoda R. Inhibition of cytochrome P450 activities by extracts of *Hyptis verticillata* Jacq.: assessment for potential HERB-drug interactions. Molecules (Basel, Switzerland), 2018; 23(2):430.

Reddy KS, Kumar AS, Ganapaty, S. Evaluation of anti-ulcer activity of Paederia foetida root extracts in experimentally induced gastric ulcer in rats. Int J Res Ayur Pharm, 2011; 2(5): 1556-1559

Roy R, Bagchi GK, Samanta J, Kang SS. Investigation of hepatoprotective activity of aqueous and ethanolic extracts of Paederiafoetida on paracetamol induced hepatotoxicity in animal model. Int J Sci Eng Res, 2017; 8(8):1691- 1696

Rozenberg R, Ruibal-Mendieta NL, Petitjean G, Cani P, Delacroix DL, Delzenne NM, Meurens M, Quetin-Leclercq J, Habib-Jiwan JL. Phytosterol analysis and characterization in spelt (*Triticum aestivum* ssp. spelta L.) and wheat (*T. aestivum* L.) lipids by LC/APCI-MS. J Cereal Sci, 2003; 38(2):189–97.

Teschke R, Frenzel C, Glass X, Schulze J, Eickhoff, A. Herbal hepatotoxicity: a critical review. Brit J Clin Pharmacol, 2013; 75(3):630–6.

Turkmenoglu FP, Kutsal YG, Dolgun AB, Diker Y, Baydar T. Evaluation of herbal product use and possible herb-drug interactions in Turkish elderly. Compl Ther Clin Pract, 2016; 23:46-51.

Uddin B, Nahar T, Basunia M, Hossain S. *Paederia foetida* protects liver against hepatotoxin-induced oxidative damage. Adv Biol Res, 2011; 5:267–72.

Upadhyaya S. Screening of phytochemicals, nutritional status, antioxidant and antimicrobial activity of *Paederia foetida* Linn. from different localities of Assam, India. J Pharm Res, 2013; 7(1):139–41.

Wienkers LC, Heath TG. Predicting in vivo drug interactions from in vitro drug discovery data. Nat Rev Drug Discov, 2005; 4(10):825–33.

Woldemeskel M. Toxicologic pathology of the reproductive system. In: Gupta RC (ed.). Reproductive and developmental toxicology. 2nd edition, Academic Press, Cambridge, MA, pp 1209–41, 2017.

Xing Y, Yan J, Niu Y. PXR: a center of transcriptional regulation in cancer. Acta Pharmaceutica Sinica B, 2020; 10(2):197–206.

Xu C, Li CY, Kong AN. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res, 2005; 28(3):249–68.

Zhou H, Yang L, Wang C, Li Z, Ouyang Z, Shan M, Gu J, Wei Y. Gene knockout reduces the metabolism and efficacy of venlafaxine in rats. Drug Metab Disposi, 2019; 47(12):1425–32.

How to cite this article:
Pouyfung P, Kuraeiad S, Yimthiang S, Khamphaya T. Long-term oral administration of *Paederia foetida* decreases cytochrome P450 mRNA expression: The predictive approaches in a rat model. J Appl Pharm Sci, 2023; 13(02):021–028.