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

Phisit Pouyfung1,2, Saruda Kuraeiad3,4, Supabhorn Yimthiang1,2, Tanaporn Khamphaya1,2,*

1Occupational Health and Safety, School of Public Health, Walailak University, Nakhon Si Thammarat, Thailand.
2Graduate Studies Program, School of Public Health, Walailak University, Nakhon Si Thammarat, Thailand.
3Medical Technology, School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat, Thailand.
4Research Excellence Center for Innovation and Health Product, Walailak University, Nakhon Si Thammarat, Thailand.

**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...
of long-term effects of *P. foetida* accumulation in rat organs and toxicity associated with herbs (Turkenmešoğlu 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 (a) 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 drug-metabolizing enzymes in rats. To predict and avoid herb-drug interaction, the predominant forms of cytochrome p450 in drug-metabolizing enzymes in rats.

**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 ionization (ESI) (Bruker Daltonics GmbH, Bremen, Germany).

After xenobiotics enter the human body, they undergo first-pass 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.

**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% 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 RNase 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_g1), 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle control</th>
<th>50</th>
<th>100</th>
<th>500</th>
<th>1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW (g)</td>
<td>226.2 ± 2.2</td>
<td>228.4 ± 3.5</td>
<td>228.4 ± 3.1</td>
<td>226.6 ± 3.6</td>
<td>225.0 ± 2.9</td>
</tr>
<tr>
<td>Final BW (g)</td>
<td>449.2 ± 5.1</td>
<td>457.1 ± 19.4</td>
<td>452.4 ± 9.2</td>
<td>456.9 ± 4.6</td>
<td>454.5 ± 12.5</td>
</tr>
<tr>
<td>BW gain (g)</td>
<td>223.0 ± 5.5</td>
<td>228.7 ± 18.8</td>
<td>224.0 ± 9.1</td>
<td>230.3 ± 6.2</td>
<td>229.5 ± 11.6</td>
</tr>
<tr>
<td>% relative</td>
<td>100.0 ± 2.5</td>
<td>102.6 ± 8.4</td>
<td>100.5 ± 4.1</td>
<td>103.3 ± 2.8</td>
<td>102.9 ± 5.2</td>
</tr>
</tbody>
</table>

There is no difference between groups (p > 0.05).
to various forms of toxic plants. To explore the safety use of *Paederia 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 *Paederia foetida* in rats, including the following: i) the administration of 500 mg/kg (p.o.) *Paederia foetida* methanal extract for 28 days protects the rat’s

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

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

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

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

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

There is no difference between groups (*p* > 0.05).
kidneys from alloxan-induced diabetic renal injury (Borgohain et al., 2017); ii) 200 mg/kg BW *P. foetida* ethanol 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 CCl₄-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 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).
Pouyfung et al. 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 the mRNA expression levels 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 Cyp3a1, 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, epifriedelino1, 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 molar 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

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.
REFERENCES
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. Phytochemistry, 2017; 36:37–49.