Effect of *Phyllanthus amarus* Schum. & Thonn. and Its Protective Mechanism on Paracetamol Hepatotoxicity in Rats

Malinee Wongnawa, Peerarat Thaina and Nisita Bumrungwong  
Department of Pharmacology  
Faculty of Science  
Prince of Songkla University  
Hat-Yai, 90112  
Thailand

Anupong Nitiruangjarat and Apichat Muso  
Department of Pathology  
Faculty of Medicine  
Prince of Songkla University  
Hat-Yai, 90112  
Thailand

Vipavadee Prasartthong  
Department of Biomedical Science  
Faculty of Medicine  
Prince of Songkla University  
Hat-Yai, 90112  
Thailand

**Keywords:** antioxidant activity, cytochrome P450, Luk-tai-bai, paracetamol metabolism, medicinal plant, glutathione

**Abstract**  
The hepatoprotective effect of *Phyllanthus amarus* Schum. & Thonn. was studied on paracetamol hepatotoxicity in rats by monitoring serum transaminase (SGOT and SGPT), alkaline phosphatase (ALP) and bilirubin as well as by histopathological examination of liver. Furthermore, the hepatoprotective mechanisms were investigated by determining the amount of paracetamol and its metabolites (glucuronide, sulfate, cysteine and mercapturic acid conjugates) in urine and pentobarbital-induced sleeping time to indicate the inhibition on cytochrome P450. The involvement of glutathione was evaluated by determining hepatic reduced glutathione. Its antioxidant activity was estimated by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Hot water extracts of *P. amarus* (0.8, 1.6 or 3.2 g/kg) were orally administered b.i.d. for 7 d prior, 2 d after, or 7 d prior and 2 d after single oral dose of paracetamol (3 g/kg). The results showed that the extract at 1.6 and 3.2 g/kg decreased the paracetamol-induced hepatotoxicity as indicated by the decrease in SGOT and SGPT, bilirubin and histopathological score while the ALP did not change. It is evident that the hepatoprotective mechanism of this plant was neither related to inhibition on cytochrome P450, nor induction on sulfate and glucuronide conjugation pathways of paracetamol, but partly due to the antioxidant activity and the protective effect on the decrease of hepatic reduced glutathione. These results support the value of *P. amarus*, which has been used in folk medicine for the treatment of toxic liver diseases.

**INTRODUCTION**  
*Luk-tai-bai* is the Thai common name for *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae). It is a small herb used in Thai folk medicine for treatment of fever, jaundice, ascites, hemorrhoid and diabetes (Pongboonrod, 1979). Apart from these medicinal uses, there are reports showing anti-hepatitis B virus effect (Thyagarajan et al., 1988), hypoglycemic effect (Moshi et al., 1997), antinociceptive effect (Santos et al., 2000) and the increase in life span of rats with hepatocellular carcinoma (Rajeshkumar and Kuttan, 2000).  
Paracetamol is a popular analgesic and antipyretic agent. Its antipyretic and analgesic properties have been widely abused. Indiscriminate ingestion can lead to accidental poisoning and potentially lethal hepatotoxicity (Prescott et al., 1971).  
Lignans from *luk-tai-bai* (phyllanthin and hypophyllanthin) showed protective...
effect on CCl4-induced hepatotoxicity in isolated hepatocytes (Syamasundar et al., 1985). However, the protective effect of luk-tai-bai on paracetamol-induced hepatotoxicity has not been evaluated. In this study, we determined the protective effect of luk-tai-bai hot water extract on paracetamol-induced hepatotoxicity in rats and investigated its mechanism by studying the effect on the metabolic pathway of paracetamol. The effect on hepatic reduced glutathione and radical scavenging activity were also evaluated.

MATERIALS AND METHODS

Preparation of the Plant Extract

The whole plant, collected in Hat-Yai, Songkla, Thailand, was identified by Purintavoragul (Department of Botany, Faculty of Science, Prince of Songkla University). A voucher specimen (number 111555) was deposited in the PSU Herbarium. Dried plant was minced and boiled with water for 15 min and then filtered. The filtrate was centrifuged, the supernatant filtered and adjusted to 1 g/mL.

Test Animals

Male Wistar rats (200-250 g) from the animal center, Faculty of Science, Prince of Songkla University, were housed at 22±3°C with a 12 h light-dark cycle and fed with pelleted diet and water ad libitum. The study was approved by the Ethics Committee on Animal Experiment, Faculty of Science, Prince of Songkla University, Thailand.

Experimental Procedures

1. Hepatoprotective Study. Rats were divided into 10 groups. Group I was orally administered with 50% sucrose (10 mL/kg) as normal control. Group II was given single oral dose of paracetamol (3 g/kg) suspension (in 50% sucrose). Groups III-V were given luk-tai-bai extracts (0.8, 1.6, 3.2 g/kg) orally b.i.d. for 7 d prior to paracetamol dosing. Group VI-VIII were given luk-tai-bai extracts (0.8, 1.6, 3.2 g/kg) orally b.i.d. for 2 d after paracetamol dosing. Group IX-X were given luk-tai-bai extracts (1.6, 3.2 g/kg) orally b.i.d. for 7 d prior to and 2 consecutive days after paracetamol dosing.

2. Assay of Serum GOT, GPT, ALP and Bilirubin. Forty-eight hours after paracetamol intoxication, the rats were sacrificed and blood was collected from orbital plexus. The activity of serum glutamate-oxaloacetate-transaminase (SGOT), serum glutamate-pyruvate-transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin were measured by an Automated Analyzer (HITACHI 717, Boehringer Mannheim, Germany).

3. Histopathological Study of Liver. The hepatic tissue was fixed in 10% formalin and stained with hematoxylin and eosin dye. The histological sections were examined under a light microscope and the extent of necrosis was graded as: normal (0), minimal centrilobular necrosis (+1), extensive necrosis confined to centrilobular region (+2), necrosis extending from central to mid zone or further to portal triad (+3).

4. Pentobarbital-induced Sleeping Time. Rats were divided into 6 groups: Group I received distilled water; Group II was pretreated with phenobarbital sodium (60 mg/kg, i.p. daily) for 4 days; Group III was 30 min pretreated with SKF-525A (15 mg/kg, i.p.); Groups IV-VI were pretreated with luk-tai-bai extracts (0.8, 1.6, 3.2 g/kg) b.i.d. orally for 7 d; Pentobarbital (35 mg/kg, i.p.) was injected and the sleeping time was recorded.

5. Assay of Paracetamol and Its Metabolites in Urine. Rats were divided into two groups. Group I was given single oral dose of paracetamol (3 g/kg). Group II was given luk-tai-bai extract (3.2 g/kg) b.i.d. for 7 d prior to paracetamol dosing. The urine was collected during 24 h after paracetamol dosing. The concentration of paracetamol and its metabolites (glucuronide, sulfate, cysteine and mercapturic acid conjugates) were determined by HPLC as given by Miners et al. (1984).

6. Assay of Glutathione in Liver. Rats were divided into 4 groups. Group I was orally given 50% sucrose as control. Group II was given single oral dose of paracetamol (3 g/kg) suspension. Group III was given luk-tai-bai extract (3.2 g/kg) orally b.i.d. for 7 d. Group IV was given luk-tai-bai extract (3.2 g/kg) orally b.i.d. for 7 d prior to paracetamol
dosing. Six hours after paracetamol intoxication, the animals were sacrificed. Hepatic reduced glutathione was determined as described by Mitchell et al. (1973).

7. Assay of Radical Scavenging Activity. Radical scavenging activity was measured by a decrease in absorbance at 520 nm of a methanol solution of colored DPPH (1,1-diphenyl-2-picrylhydrazyl) as described by Hatano et al. (1989). Luk-tai-bai aqueous extracts (100-300 µg/mL) were mixed (1:1) with DPPH solution (60 µM). The absorbances were measured after 30 min. EC50 was calculated from % inhibition. BHT (butylated hydroxytoluene) was used as a standard.

Chemicals
Paracetamol (A5000), paracetamol glucuronide (A4438), phenobarbital sodium (P5178), pentobarbital (P3761), SKF-525A (P1061), DPPH (1,1-diphenyl-2-picrylhydrazyl) (D9139), BHT (butylated hydroxytoluene) (T5500) and Ellman’s disulfide reagent [bis-(3-carboxy-4-nitro-phenyl disulfide)] (D8130) were purchased from the Sigma (St Louis, MO). All other chemicals were of analytical grade.

Statistical Analysis
Values were expressed as mean±S.E.M. The biochemical parameters were statistically assessed by one-way analysis of variance (ANOVA). The difference between groups was evaluated by Student’s t-test. Liver histopathological data were analyzed by Kruskal-Wallis test followed by Newman-Keuls test (P ≤ 0.05).

RESULTS
Hepatoprotective Effect on Paracetamol Induced Hepatotoxicity
A single oral dose of paracetamol (3 g/kg) induced a significant increase in SGOT, SGPT, ALP, bilirubin and histopathological score in comparison with normal controls (Table 1). Treatment of rats with luk-tai-bai aqueous extracts at the dose of 1.6, 3.2 g/kg for 7 d before administration of paracetamol exhibited a significant reduction of the biochemical parameters. The SGOT and SGPT levels were reduced by about 50%, while serum bilirubin were down about 20%. Post-treatment with luk-tai-bai showed less effect. The treatment for 7 d before and 2 d after paracetamol dosing markedly reduced SGOT, SGPT and bilirubin by about 70%, 60% and 40%, respectively. The histopathological score was correlated with the changes of serum markers, while the ALP was unchanged.

Effect on Pentobarbital Sleeping Time
Pentobarbital sleeping time in the rats pretreated with phenobarbital was shortened and those of rats pretreated with SKF-525A was prolonged, whereas pretreatment with luk-tai-bai extracts did not prolong the sleeping time, as compared with the control group (Fig. 1).

Effect on Paracetamol Metabolism
The proportions of paracetamol and its metabolites: glucuronide, sulfate, cysteine and mercapturic acid conjugates excreted in urine in luk-tai-bai pretreated group were not significantly different from the control group (Fig. 2).

Effect on Hepatic Reduced Glutathione
Paracetamol (3 g/kg) caused decrease in hepatic GSH content at 6 h post-dosing when compared with control group (Table 2). Luk-tai-bai extract (3.2 g/kg b.i.d. orally for 7 d) slightly increased GSH. The depletion of GSH in the rats treated with paracetamol was partially prevented by luk-tai-bai pretreatment.

DPPH Radical Scavenging Activity
It was found that the aqueous extract of luk-tai-bai exhibited a maximum DPPH radical scavenging activity of 88% at the concentration of 150 µg/mL with EC50 value of
45 µg/mL, which is approximately two times less than the standard BHT (EC₅₀ = 20 µg/mL).

**DISCUSSION**

Liver injury induced by paracetamol is commonly used as a model for the screening of hepatoprotective drugs. Raised serum enzyme (SGOT, SGPT, ALP) levels in intoxicated rats can be attributed to the damaged structural integrity of the liver, because these enzymes are located in cytoplasm and are released into circulation after cellular damage (Sallie et al., 1991). This study showed that pretreatment with *luk-tai-bai* extract can protect against paracetamol-induced hepatotoxicity in rats as judged from the decrease in SGOT, SGPT and serum bilirubin. The results were also confirmed by histopathological study.

The mechanism of cell damage appears to be mediated by the metabolic activation of paracetamol, via cytochrome P450 (CYP) activity especially CYP 2E1, to a highly reactive toxic metabolite (N-acetyl-p-benzoquinoneimine, NAPQI), which is able to deplete hepatocellular glutathione (GSH) and to covalently bind to cellular macromolecule, leading to cellular damage (Halliwell and Gutteridge, 1999). Moreover, the oxidizing ability of this metabolite is also responsible for toxicity. GSH, the predominant intracellular non-protein sulfhydryl present in the cytosol, is a strong nucleophile, able to react with electrophilic such as NAPQI, and it is a reducing agent that contributes to the protection of cells against oxidative stress. The concentration of intracellular GSH, therefore, is a key determinant of the extent of paracetamol-induced hepatic injury. The glutathione-derived conjugates (cysteine and mercapturic acid conjugates) are then excreted in urine in proportion to the toxic metabolite formed. However, the sulfation and glucuronidation are the major pathways of paracetamol metabolism (Miners et al., 1984).

Pentobarbital is metabolized by CYP, thus any drug with an inhibitory effect on CYP is expected to prolong pentobarbital-sleeping time (Fugimoto et al., 1960). The finding that *luk-tai-bai* extract did not prolong pentobarbital-sleeping time, while phenobarbital (a CYP inducer) shortened and SKF-525A (a CYP inhibitor) prolonged the sleeping time, suggests that it is devoid of any inhibitory effect on CYP. This result was confirmed by the finding that the urine proportion of cysteine and mercapturic acid conjugates did not change in *luk-tai-bai* pretreatment, compared with control. These findings suggest that *luk-tai-bai* extract did not inhibit CYP activity, which is responsible for paracetamol metabolism to produce toxic metabolite (Halliwell and Gutteridge, 1999). Moreover, *luk-tai-bai* extract did not induce paracetamol sulfation or glucuronide conjugation. These results suggest that the hepatoprotective effect of *luk-tai-bai* was not related to the effect on the metabolic pathway of paracetamol, but mediated perhaps through other mechanisms. Administration of paracetamol to rats caused a severe depletion of hepatic GSH, which can be attributed to GSH consumption by glutathione transferase for metabolite conjugation, and to GSH oxidation for the defense against the produced oxidative stress (Halliwell and Gutteridge, 1999). Our results showed that *luk-tai-bai* extract alone caused a slight increase in GSH. The GSH depletion in paracetamol intoxication was partially prevented as well in rats pretreated with *luk-tai-bai*. This activity might be one of the hepatoprotective mechanisms of *luk-tai-bai*.

The radical scavenging activity of *luk-tai-bai* may be due to active compounds containing polyphenol group such as lignans (phyllanthin and hypophyllanthin), flavonoids (quercetin and astragalin), ellagittannins (amarinic acid) and hydrolysable tannins (phyllanthiisin D and amarin) (Rajeshkumar and Kuttan, 2000).

In conclusion, *luk-tai-bai* aqueous extract exhibited the hepatoprotective activity on paracetamol intoxication. The hepatoprotective mechanism was not related to the inhibition of cytochrome P450 or induction of sulfate and glucuronide conjugation, but partly due to the radical scavenging activity and the protective effect on GSH depletion. However, other possible mechanisms in hepatoprotective effects such as induction of glutathione linked detoxification system (glutathione-s-transferase, glutathione
peroxidase, glutathione reductase) and antioxidant enzymes (superoxide dismutase, catalase) need further investigation. These results support the value of *luk-tai-bai*, as used in Thai folk medicine for treatment of liver disease.

**ACKNOWLEDGEMENTS**

This research was supported by grants from the Ministry of Public Health and Prince of Songkla University, Thailand. We are grateful to Assistant Professor Choathip Purintavoragul for identification of the plant.

**Literature Cited**


Table 1. Effects of paracetamol and *P. amarus* aqueous extract on serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkali phosphatase (ALP), bilirubin and histopathological change in rats (mean±S.E.M., n=7-19).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg%)</th>
<th>Histopathological score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>106±5</td>
<td>53±2</td>
<td>282±19</td>
<td>0.04±0.01</td>
<td>0</td>
</tr>
<tr>
<td>Paracetamol 3 g/kg</td>
<td>3569±615*</td>
<td>2622±460*</td>
<td>353±27*</td>
<td>0.24±0.04*</td>
<td>2.9±0.1*</td>
</tr>
<tr>
<td><em>P. amarus</em> extract 0.8 g/kg</td>
<td>3116±757</td>
<td>1796±333</td>
<td>380±31</td>
<td>0.22±0.02</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>1.6 g/kg</td>
<td>1660±489a</td>
<td>1239±351a</td>
<td>351±31</td>
<td>0.18±0.03</td>
<td>2.3±0.2a</td>
</tr>
<tr>
<td>3.2 g/kg</td>
<td>2111±810a</td>
<td>1106±350a</td>
<td>328±21</td>
<td>0.19±0.05</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>b.i.d. for 7 days before paracetamol <em>P. amarus</em> extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8 g/kg</td>
<td>3092±929a</td>
<td>2205±753a</td>
<td>379±45</td>
<td>0.22±0.04</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>1.6 g/kg</td>
<td>2162±781a</td>
<td>1390±383a</td>
<td>443±42</td>
<td>0.25±0.06</td>
<td>2.2±0.4a</td>
</tr>
<tr>
<td>3.2 g/kg</td>
<td>2416±649a</td>
<td>1546±493a</td>
<td>349±16</td>
<td>0.23±0.05</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>b.i.d. for 2 days after paracetamol <em>P. amarus</em> extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.6 g/kg</td>
<td>921±265a</td>
<td>994±334a</td>
<td>293±26</td>
<td>0.14±0.02</td>
<td>2.3±0.3a</td>
</tr>
<tr>
<td>3.2 g/kg</td>
<td>1210±391a</td>
<td>1538±536a</td>
<td>303±40</td>
<td>0.18±0.05</td>
<td>2.0±0.4a</td>
</tr>
</tbody>
</table>

* significantly different from control group (*P* < 0.05)
a significantly different from paracetamol group (*P* < 0.05)

Table 2. Effect of *P. amarus* extract and paracetamol on hepatic reduced glutathione in rats (mean±S.E.M., n=6-9).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g</td>
</tr>
<tr>
<td>Control (50% sucrose)</td>
<td>7.20±0.25</td>
</tr>
<tr>
<td>Paracetamol (3 g/kg) single oral dose</td>
<td>1.91±0.15*</td>
</tr>
<tr>
<td><em>P. amarus</em> extract (3.2 g/kg) b.i.d. orally for 7 d</td>
<td>7.95±0.25*</td>
</tr>
<tr>
<td><em>P. amarus</em> extract (3.2 g/kg) b.i.d. orally for 7 d before paracetamol (3 g/kg)</td>
<td>2.53±0.24a</td>
</tr>
</tbody>
</table>

* significantly different from control group (*P* < 0.05)
a significantly different from paracetamol group (*P* < 0.05)
**Figures**

**Fig. 1.** Effects of *P. amarus* extract, phenobarbital and SKF-525A pretreatment on pentobarbital-induced sleeping time in rats (mean ± S.E.M., n = 9-12). * significantly different from control.

**Fig. 2.** Effects of *P. amarus* on urinary paracetamol (P) and its metabolites (G = glucuronide, S = sulfate, C = cysteine, M = mercapturic acid conjugates) after a 3 g/kg dose of paracetamol in rats (mean ± S.E.M., n = 10).