

# The influence of bile acids homeostasis by cryptotanshinone-containing herbs

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## Abstract

**Background:** Herbs might affect the homeostasis of bile acids through influence of multiple metabolic pathways of bile acids.

**Objective:** To investigate the inhibition of cryptotanshinone towards the glucuronidation of LCA, trying to indicate the possible influence of cryptotanshinone-containing herbs towards the homeostasis of bile acids.

**Methods:** The LCA-3-glucuronidation and LCA-24-glucuronidation reaction was monitored by LC-MS.

**Results:** Initial screening showed that 100  $\mu$ M of cryptotanshinone inhibited LCA-24-glucuronidation and LCA-3-glucuronidation reaction activity by 82.6% and 79.1%, respectively. This kind of inhibition behaviour exerted cryptotanshinone concentrations-dependent and LCA concentrations-independent inhibition behaviour.

**Conclusion:** All these data indicated the possibility of cryptotanshinone's influence towards bile acids metabolism and homeostasis of bile acids.

**Keywords:** herbs, lithocholic acid (LCA), homeostasis

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## Introduction

Bile acids, initiated by CYP7A1-catalyzed cholesterol oxidation in liver, exert an important role in the solubilization, absorption, and transportation of dietary lipids in the intestine<sup>1</sup>. The most abundant bile acids in human are consisted of cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), and lithocholic acid (LCA)<sup>1</sup>. The homeostasis of bile acids can be tightly regulated through feed-back and feed-forward regulation pathways. Bile acids exert their toxicity towards cells at high concentrations, and the accumulation of bile acids can induce the severe damage towards liver cells<sup>2</sup>.

Bile acids have been reported to induce cell injury through many mechanisms, including the damage to the plasma membrane, oxidative stress, apoptosis, and inflammation<sup>3,4</sup>. Among the bile acids, LCA has been commonly considered as the most toxic bile acid which has widely used as the inducer of cholestasis<sup>5</sup>. Herbs can affect the metabolism of bile acids. For example, glycyrrhizin exhibited protective role towards LCA-induced liver injury through pregnane

X receptor (PXR) mediated-transcription regulation of cytochrome 3A which has been regarded as the most important enzyme involved in the metabolism of LCA<sup>6</sup>.

Besides CYP3A-catalyzed metabolism of LCA, glucuronidation of LCA has been regarded as another important metabolic pathway of LCA. The LCA-3-glucuronide (LCA-3G) and LCA-24-glucuronide (LCA-24G) are the major glucuronides<sup>7</sup>. The inhibition of the glucuronidation of LCA might increase the toxicity induced by LCA. To date, no report was given on the influence of herbs on the glucuronidation of LCA. Danshen is a perennial plant in the genus *Salvia*, highly valued for its roots in traditional Chinese medicine. The present study aims to evaluate the inhibition of danshen's major ingredient cryptotanshinone as an example towards the glucuronidation of LCA.

## Materials and Methods

### Reagents

Lithocholic acid (LCA), uridine-5'-diphosphoglucuronic acid (UDPGA) (trisodium salt), Tris-HCl, alamethicin from *Trichoderma viride* (purity $\geq$ 98%) were purchased from Sigma-Aldrich (St Louis, MO). Pooled human liver microsomes (pHLMs) were purchased from BD Gentest. Cryptotanshinone was obtained from Shanghai Tauto Biotech Co. (Shanghai, China).

### Evaluation of inhibitory effect of cryptotanshinone towards the LCA glucuronidation reaction

As previously described<sup>8</sup>, the incubation mixture (total volume=100  $\mu$ L) contained 50 mM Tris-HCl (pH=7.4),

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10 mM MgCl<sub>2</sub>, 25 µg of alamethicin, 0.5 mg/ml HLMs, and LCA (100 µM). All the glucuronidation incubations were performed under 37 °C for 1h. 200 µL ice-cold methanol was used to terminate the reaction. After centrifugation at 12000 rpm, the aliquots (10 µL) of supernatant were used to undergo UPLC-MS analysis. For UPLC analysis, the Shim-pack XR-ODS II column (75\*2.0mm,2.2µm) was used. The flow rate was set as 0.3 ml/min. The eluents were methanol (A) and 0.2% formic acid (B) with the following gradients: 0-4 min, 35-70% A; 4-8 min, 70-80% A; 8-12min, 80-95% A; 12-15 min, 35% A. The negative mode was used to monitor LCA and its glucuronides. The ions [M-H]<sup>-</sup> 375.1 and 551.1 were selected to monitor LCA and its glucuronides, respectively.

### Results

Under recent analysis condition, LCA was eluted at 2.3 min, and its glucuronides LCA-3-glucuronide (LCA-3-G) and LCA-24-glucuronide (LCA-24-G) were eluted at 1.15 min and 1.35 min, respectively. 100 µM of cryptotanshinone was firstly employed to investigate the inhibition towards LCA glucuronidation. 82.6% inhibition was detected for LCA-24-glucuronidation reaction (Fig. 1), and 79.1% activity of LCA-3-glucuronidation activity was inhibited (Fig. 3). Furthermore, the inhibition capability of various concentrations of cryptotanshinone towards the glucuronidation metabolism of LCA at different concentrations was investigated. Significant dose-dependent inhibition behaviour was observed for cryptotanshinone (Fig. 2), however, the concentration-dependent inhibition behaviour for the LCA concentration was not observed (Fig. 4).

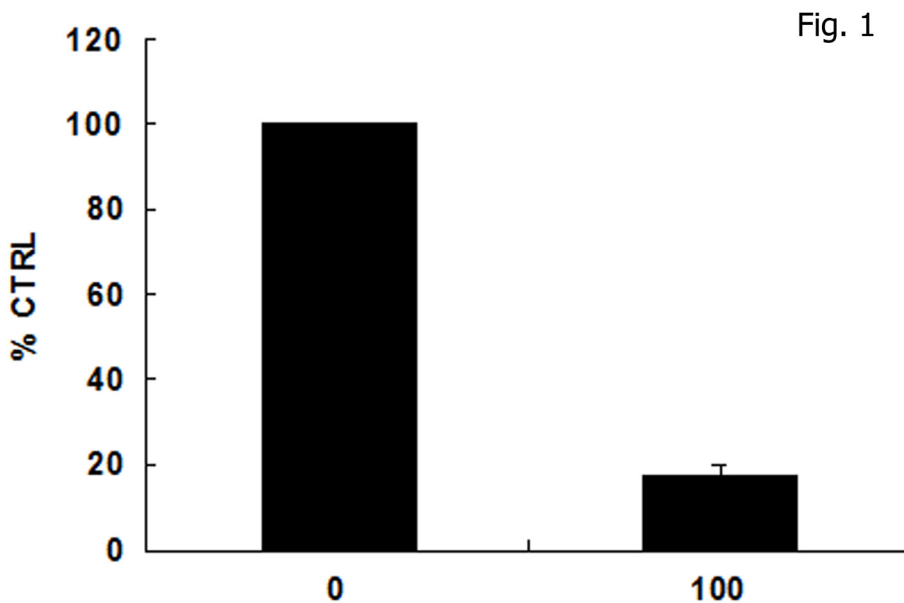


Fig. 1

Fig. 1 The initial screening of cryptotanshinone's inhibitory capability towards the LCA-24-glucuronidation reaction activity. 50 µM of LCA was used, and 100 µM of cryptotanshinone was used. The data were given as mean plus S.D..

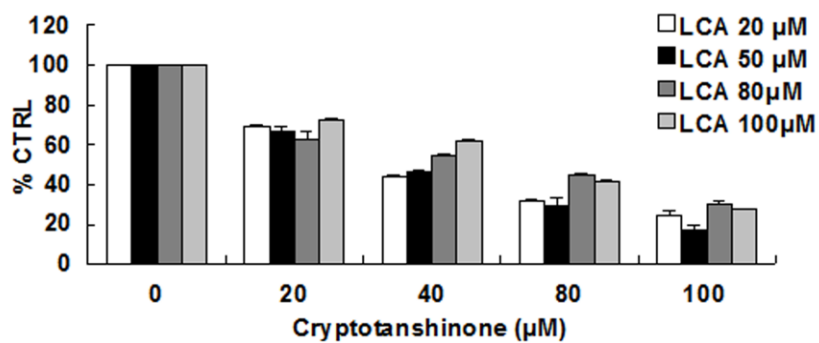


Fig. 2

Fig. 2 Different inhibitory potential of various concentrations of cryptotanshinone towards the LCA-24-glucuronidation reaction activity. Various concentrations of cryptotanshinone (0, 20, 40, 80 and 100 μM) and LCA (20, 50, 50, and 100 μM) were used.

Fig. 3

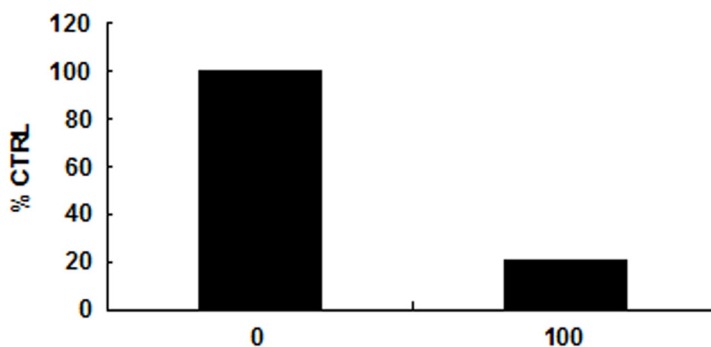


Fig. 3 The initial screening of cryptotanshinone's inhibitory capability towards the LCA-3-glucuronidation reaction activity. 50 μM of LCA was used, and 100 μM of cryptotanshinone was used. The data were given as mean plus S.D..

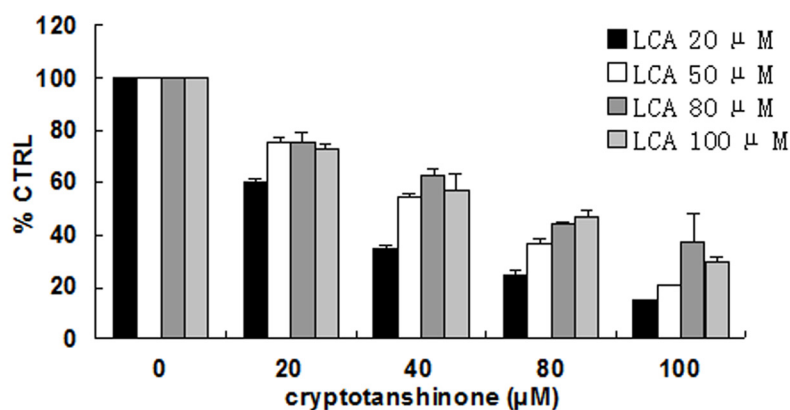


Fig. 4

Fig. 4 Different inhibitory potential of various concentrations of cryptotanshinone towards the LCA-3-glucuronidation reaction activity. Various concentrations of cryptotanshinone (0, 20, 40, 80 and 100  $\mu\text{M}$ ) and LCA (20, 50, 50, and 100  $\mu\text{M}$ ) were used.

## Discussion

Multiple metabolic pathways catalyzed by enzymes contributed to the homeostasis of bile acids. The xenobiotics can affect the serum level of bile acids through influence of all these pathways, including synthesis and detoxification pathways. For example, herbs can up-regulate the activity of CYP7A1 which accelerate the synthesis of bile acids<sup>9</sup>. Glycyrrhizin, a major ingredient of licorice, can increase the detoxification process of LCA through accelerating CYP3A-catalyzed metabolism of LCA<sup>6</sup>.

The present study focused on the glucuronidation detoxification of bile acids, using LCA as a typical substrate. LCA can undergo efficient glucuronidation process in 3-OH and 24-OH. Therefore, the inhibition potential of cryptotanshinone towards both LCA-3-glucuronidation and LCA-24-glucuronidation reactions was investigated in the present study. Strong inhibition of cryptotanshinone towards LCA-3-glucuronidation and LCA-24-glucuronidation was demonstrated, and this kind of inhibition was demonstrated to be cryptotanshinone concentrations-dependent and LCA concentrations-independent. This result showed the possible disturbance of cryptotanshinone towards the metabolism of LCA, indicating the possible influence of cryptotanshinone towards the homeostasis of bile acids. It should be noted that the enzymes involved in the metabolism of LCA (e.g. UGT1A3) were also involved

in the metabolism of other bile acids (e.g., chenodeoxycholic acid)<sup>10</sup>. Therefore, danshen might also disturb the homeostasis of bile acids through inhibition of other bile acids' metabolism.

It should be noted that the present study results should be explained in the context of herbal complexity. Many other components in herbs might complicate the final influence of herbs towards the homeostasis of bile acids. Additionally, the long administration of herbs might also up-regulate the enzymes involved in the glucuronidation of LCA through important nuclear receptors (e.g., farnesoid X receptor (FXR), peroxisome proliferator-activated receptor alpha (PPARalpha), etc.). For example, nuclear receptor farnesoid X receptor (FXR) has been reported to activate the activity of UGT1A3 which is a key enzyme involved in the glucuronidation of LCA<sup>10</sup>.

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