

## EFFECTS OF *CURCUMA COMOSA* EXTRACTS ON PHASE II DRUG-METABOLIZING ENZYMES IN RAT LIVERS

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**ABSTRACT:** On the process of drug research and development, drug interaction study is required either preclinically or clinically. Modulation of drug metabolism is one of the etiology of drug interaction resulting in reduction of drug efficacy or in the other hand, increase of drug toxicity. This interaction is mostly associated with induction or inhibition at phase I or phase II metabolism. The objective of this study was to investigate effects of *Curcuma comosa* hexane and ethanolic extracts on phase II drug metabolizing enzymes involving in drug metabolism such as UDP-glucuronosyltransferase (UDPGT), sulfotransferase (SULT), glutathione S-transferase (GST), and NAD(P)H quinoneoxidoreductase (NQOR) in rat livers. Fifty male Wistar rats were randomly divided into 5 groups of 10 rats each. Rats in the control group were given corn oil at 1 ml/kg/day whereas rats in group 2 and 3 received *C. comosa* hexane extract (which was dissolved in corn oil) orally at dosages of 250 and 500 mg/kg/day. Rats in group 4 and 5 received *C. comosa* ethanolic extract orally at dosages of 250 and 500 mg/kg/day. At the end of the extract administration (30 days), rats were anesthetized. Microsomes and cytosols were prepared from the livers for enzyme activity assays. The results showed that *C. comosa* hexane extracts at the dosages of 250 and 500 mg/kg/day significantly increased UDPGT activity whereas NQOR activity was significantly increased when *C. comosa* hexane extract at 500 mg/kg/day was given. Both extracts did not affect SULT and GST activities. These results indicated that co-administration of *C. comosa* hexane extract with some drugs that are metabolized by UDPGT and/or NQOR may affect drug level resulting in reduction of efficacy or increase of adverse drug reaction. In addition, an increase of UDPGT and NQOR activities by *C. comosa* indicated a potential benefit of this plant for a decrease of risks from chemical-induced carcinogenesis and/or mutagenesis. Effects of *C. comosa* extracts on other phase II drug metabolizing enzymes should be further explored.

**Keyword:** *Curcuma comosa*, drug interaction, UDP-glucuronosyltransferase, sulfotransferase, glutathione S-transferase, NAD(P)H quinoneoxidoreductase

**INTRODUCTION:** Rhizomes of *Curcuma comosa* Roxb., a plant in family ZINGIBERACEAE, have been used extensively in Thai traditional medicine as an anti-inflammatory agent particularly for the treatment of female postpartum uterine inflammation. Rhizomes of *C. comosa* comprise various chemical compounds including diaryl-heptanoids and phloracetophenone glucoside<sup>1,2</sup>. Many studies have revealed pharmacological effects of *C. comosa*, such as uterotrophic effect<sup>3</sup>, estrogenic-like effect<sup>4,5</sup>, anti-inflammatory effect<sup>6</sup>. Other pharmacological effects of *C. comosa* have also been documented such as choleretic effect<sup>7</sup>, cholesterol lowering effect<sup>7,8,9</sup>, antiatherosclerotic effect<sup>10</sup> and nematocidal effect<sup>11</sup>. These beneficial effects of *C. comosa* are promising to be developed

as an alternative medicine.

On the process of drug research and development, drug interaction study is required either preclinically or clinically<sup>11</sup>. Modulation of drug metabolism is one of the etiology of drug interaction resulting in reduction of drug efficacy or in the other hand, increase of drug toxicity. This interaction is mostly associated with induction or inhibition at phase I or phase II metabolism<sup>12</sup>. Effect of *C. comosa* on cytochrome P450, the phase I drug metabolizing enzyme, had been reported earlier<sup>13</sup>. Therefore, the aim of this study was to investigate effects of *C. comosa* hexane and ethanolic extracts on phase II enzymes involving in drug metabolism such as UDPGT, SULT, GST, and NQOR in rat livers.

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## MATERIALS AND METHODS:

### Experimental animals

Fifty adult male Wistar rats of body weight between 250-300 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. Animals were housed two per cage at the Faculty of Medicine, Srinakharinwirot University, Bangkok and acclimatized for at least a week prior to the experiment. All animals were in a controlled humidity room at a constant temperature of 25 °C and maintained on a 12-hour alternate light-dark cycle. They were allowed free access to food (CP company, Thailand) and drinking water. Rats were randomly divided into 5 groups. Each group comprised 10 rats. The experimental groups were received *C. comosa* hexane extract or ethanolic extract orally at doses of 250 and 500 mg/kg/day whereas rats in the control group were given corn oil at 1 ml/kg/day for 30 consecutive days. At the end of the treatment, animals were fasted for 12 hours before anesthetized with diethyl ether and sacrificed by cervical dislocation. The protocol of animal housing and treatment used in this study was approved by the Ethic Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Approval No. 99/2007).

### *C. comosa*

The rhizomes of *C. comosa* were collected from Nakhon Pathom, Thailand. The dried rhizome powder (30 kg) was extracted with n-hexane in a Soxhlet extractor to give a pale brownish viscous oil (1.01 kg), giving the percent yield of 3.37%. The marc was subsequently extracted with 95% ethanol, the ethanolic fraction was dried under vacuum in rotary evaporator and dried again with high vacuum pump to give a dark reddish-brown viscous oil (1.30 kg), giving the percent yield of 4.33%. Hexane and ethanolic extracts of *C. comosa* were characterized by thin layer chromatography. The major constituent of hexane extract was 1,7-diphenyl-5-hydroxy-(1*E*)-1-heptene. The major constituents of ethanolic extract were 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1*E*)-1-heptene and 7-(3, 4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene.

### Preparation of microsomes and cytosols and enzyme activity assays

Rat liver microsomes and cytosols were prepared according to the method described by Lake BG.<sup>14</sup>). Liver microsomal and cytosolic protein concentrations were determined according to the method of Lowry OH. *et al.*<sup>15</sup>). UDPGT activity was measured using *p*-nitrophenol as a substrate and UDPGA as a co-enzyme according to the method of Bock KW. *et al.*<sup>16</sup>). SULT activity was determined using 2-naphthol as a substrate as well as adenosine 3'-phosphate-5'-phosphosulfate and *p*-nitrophenylsulfate as co-enzymes according to the method of Frame LT. *et al.*<sup>17</sup>). GST activity was measured using 1-chloro-2, 4-dinitrobenzene as a substrate and the reduced form of glutathione (GSH) as a co-enzyme according to the method of Habig WH. *et al.*<sup>18</sup>). NQOR activity was determined using 2, 6-dichlorophenol-indophenol as a substrate and NADH as a co-enzyme according to the method of Ernster L.<sup>19</sup>).

### Statistical analysis

All numeric quantitative data were presented as mean  $\pm$  standard error of the mean (SEM). One-way analysis of variance (ANOVA) and Student-Newman-Keuls test were used for statistical comparison at a significant level of  $p < 0.05$ .

## RESULTS:

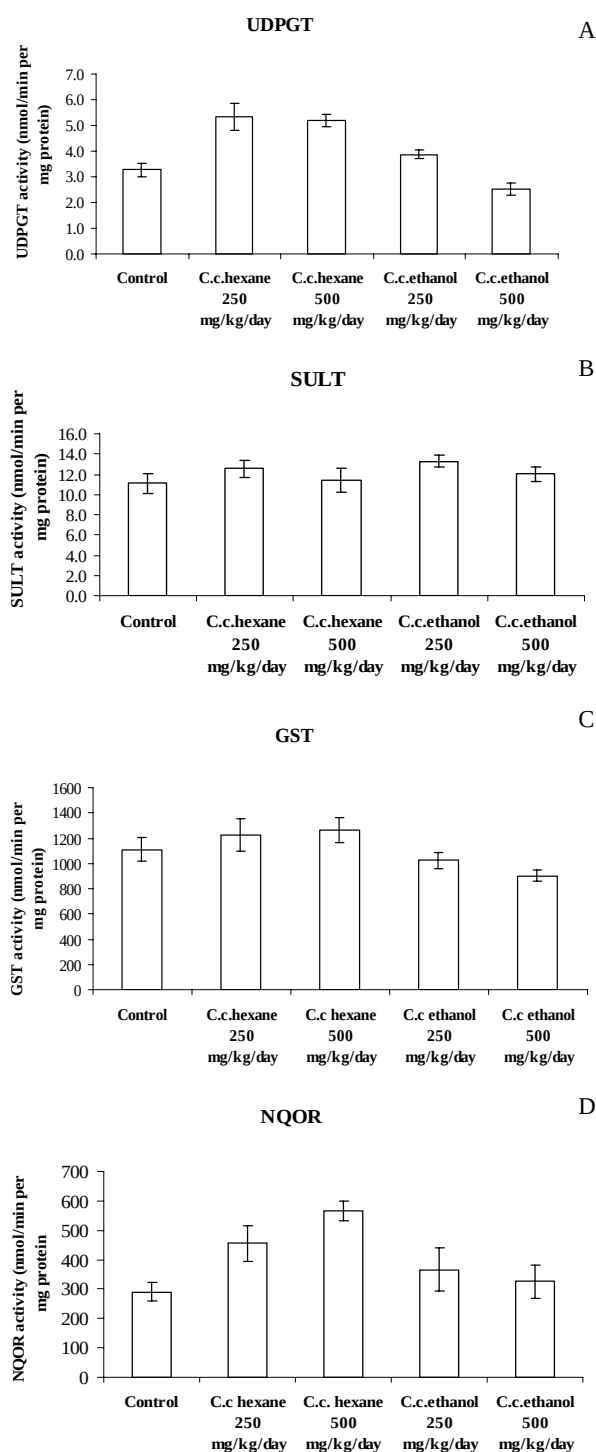
### Effect of *C. comosa* hexane extract and ethanolic extract on phase II drug metabolizing enzymes

*C. comosa* hexane extract caused an increase of UDPGT activity at both doses given to rats whereas *C. comosa* ethanolic extract did not affect this enzyme activity (Figure 1A). Both *C. comosa* hexane extract and ethanolic extract did not modulate the activities of SULT (Figure 1B) and GST (Figure 1C). For NQOR, only *C. comosa* hexane extract at the dosage of 500 mg/kg/day caused an increase of NQOR activity whereas the lower doses (250 mg/kg/day) of *C. comosa* hexane extract and both doses of *C. Comosa* ethanolic extracts did not affect the activity of this enzyme (Figure 1D).

## DISCUSSION AND CONCLUSION:

The results from this study showed that only *C. comosa* hexane extract increased the activities of UDPGT and NQOR while *C. comosa* ethanolic extract did not. Both extracts of *C. comosa* did not affect SULT and GST activities. Mostly, UDPGT plays a major role in detoxification of drugs and xenobiotics. Such drugs and xenobiotics or their metabolites that are detoxified by UDPGT include estrogens, paracetamol, morphine, naphthols, coumarin, flavonoids, etc.<sup>20</sup>. NQOR is also a detoxification enzyme in the flavin monooxygenase reactions. Drugs/xenobiotics that are detoxified by NQOR include  $\alpha$ -tocopherol, CoQ<sub>10</sub>, *p*-benzoquinone, 1,4-naphthoquinone, 2,6-dichlorophenol-indophenol, methylene blue, etc.<sup>19</sup>. Thus, an increase of UDPGT and NQOR by *C. comosa* hexane extract indicated an advantage effect of this extract in term of a decrease risk of toxicity, mutagenic and/or carcinogenic effects of many drugs and/or xenobiotics that are detoxified by this enzyme. However, an increase of UDPGT and NQOR by *C. comosa* hexane extract might be concerned in term of drug interaction resulting in a decreased efficacy of many drugs that are metabolized by these 2 enzymes.

No effects of *C. comosa* hexane and ethanolic extracts on both SULT and GST activities. Generally, SULT is a family of enzyme responsible for detoxification of many drugs/xenobiotics and/or their metabolites. Drugs/xenobiotics that are detoxified by SULT include estrogens, thyroid hormones, dopamine, eicosanoids, 2-naphthol, *p*-nitrophenol, etc.<sup>12</sup>. GST, a family of enzymes that play a major role in detoxification of most electrophilic metabolites from phase I metabolism. Reactive metabolites that are detoxified by glutathione conjugation and catalyzed by GST are aflatoxin B1-epoxide, benzpyrene diols, etc.<sup>20</sup>. Drugs that are metabolized by GST include estrogens, cortisol, paracetamol, tetracycline, ethacrynic acid, etc.<sup>12</sup>. Therefore, no effects of *C. comosa* extracts on both SULT and GST activities indicate that there are no advantage effects of these extracts regarding antimutagenic/ anticarcinogenic/protective effects against other xenobiotics that are detoxified by these enzymes.



**Figure 1** Effects of *C. comosa* hexane extract and ethanolic extract on UDPGT (A), SULT (B), GST (C) and NQOR (D) activities in rats. Rats were treated orally with corn oil (1 ml/kg/day) in the control group, *C. comosa* hexane extracts (250 or 500 mg/kg/day) and *C. comosa* ethanolic extracts (250 or 500 mg/kg/day) for 30 days. Data were presented as mean  $\pm$  SEM of 9-10 rats/group. \*  $p < 0.05$ , *C. comosa* treated group vs control group.

Also, drug interaction that is possibly caused by *C. comosa* and other drugs that are detoxified by these two enzymes are excluded. The findings of the effects of *C. comosa* on phase II enzymes found in this study provided a preliminary information in animals. Inter-animal variability of these enzymes (UDPGT, SULT, GST and NQOR) is normally existed<sup>20</sup>. Thus, interpretation of these data from animals to human should be concerned and needed a further study in human. Effects of *C. comosa* extracts on other phase II enzymes were suggested to be further explored.

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