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Original Article

Effects of lemongrass oil and citral on hepatic drug-metabolizing enzymes, oxidative stress, and acetaminophen toxicity in rats

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ABSTRACT

The essential oil from a lemongrass variety of *Cymbopogon flexuosus* [lemongrass oil (LO)] is used in various food and aroma industry products and exhibits biological activities, such as anticancer and antimicrobial activities. To investigate the effects of 200 LO (200 mg/kg) and 400 LO (400 mg/kg) and its major component, citral (240 mg/kg), on drug-metabolizing enzymes, oxidative stress, and acetaminophen toxicity in the liver, male Sprague-Dawley rats were fed a pelleted diet and administered LO or citral by gavage for 2 weeks. After 2 weeks of feeding, the effects of LO and citral on the metabolism and toxicity of acetaminophen were determined. The results showed that rats treated with 400 LO or citral had significantly reduced hepatic testosterone 6β-hydroxylation and ethoxyresorufin *O*-deethylation activities. In addition, NAD(P)H:quinone oxidoreductase 1 activity was significantly increased by citral, and UDP-glucurosyltransferase activity was significantly increased by 400 LO in the rat liver. Treatment with 400 LO or citral reduced lipid peroxidation and reactive oxygen species levels in the liver. After acetaminophen treatment, however, LO and citral treatment resulted in little or no change in plasma alanine aminotransferase activity and acetaminophen-protein adducts content in the liver. Our results indicate that LO and citral may change the activities of drug-metabolizing enzymes and reduce oxidative stress in the liver. However, LO and citral may not affect the detoxification of acetaminophen.

1. Introduction

Lemongrass is an aromatic plant belonging to the Gramineae family [1]. The essential oil isolated from the lemongrass variety (*Cymbopogon flexuosus*) is used in various food and aroma industry products. Lemongrass oil (LO) contains mainly citral [2], a natural combination of two isomeric aldehydes, namely, the isomers geranial (α-citral) and neral (β-citral) [3]. Studies have shown that LO and citral have various biological

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activities, such as anticancer and antimicrobial activities [4–7]. Moreover, citral has also been shown to be renoprotective and hepatoprotective in mice through its antioxidative and anti-inflammatory effects [8,9]. However, little is known about the effect of LO and citral on drug-metabolizing enzymes. Nakamura et al [10] indicated that citral could induce total and pi-class-specific activity of glutathione S-transferase (GST) in a normal rat liver epithelial cell line (RL34 cells). Therefore, it has been suggested that LO or citral may play a role in reducing oxidative stress and detoxifying reactions. Increasing evidence shows that phytochemicals derived from foods or herbal medicines may modulate drug-metabolizing enzymes and thereby influence the pharmacological activity of drugs and their toxicities [11]. Thus, administration of natural products enriched in phytochemicals, such as LO, should be carefully monitored when given together with therapeutic drugs.

During the phase I cytochrome P450 (CYP) oxidation reaction, the production of reactive oxygen species (ROS) can cause lipid peroxidation and oxidative stress [12]. Phase II conjugation enzymes function to eliminate the electrophiles and ROS generated by phase I reactions, thereby preventing the increase of oxidative stress [13]. Increased antioxidants and/or antioxidant enzyme activities, which are involved in the detoxification of various pathological conditions, are one of the proposed mechanisms for the therapeutic effect of herbal medicines [14]. Therefore, in addition to understanding their effects on drug metabolism, evaluating the effects of herbal medicines on the antioxidant system and oxidative stress is needed.

Acetaminophen (*N*-acetyl-p-aminophenol or APAP) is an antipyretic and analgesic drug. An overdose can induce severe hepatotoxicity as a result of the CYP-mediated metabolism of APAP into a reactive metabolite, *N*-acetyl-p-benzoquinone imine (NAPQI), which exerts its toxicity by covalent binding to cellular macromolecules such as proteins, lipids, and DNA [15]. Moreover, NAPQI also reacts with glutathione (GSH), leading to cellular GSH depletion and the production of ROS in the liver. Today, APAP overdose is the most common cause of acute hepatic failure in many countries [16].

In this study, we first evaluated the effects of LO and citral on drug-metabolizing enzymes and oxidative stress in rat livers, and then investigated whether LO and citral could protect the liver from APAP-induced hepatotoxicity in rats.

2. Materials and methods

2.1. Materials

APAP, testosterone, methoxyresorufin, resorufin, p-nitrophenol, 4-nitrocatechol, NADPH, GSH, 1-chloro-2,4-dinitrobenzene, and heparin were obtained from Sigma (St. Louis, MO, USA). 6β-Hydroxytestosterone was purchased from Ultrafine Chemicals (Manchester, UK). All other chemicals and reagents were of analytical grade and were obtained commercially. LO, the essential oil from a lemongrass variety of *C. flexuosus*, was purchased from Tai Gold Herbal Technology Co., LTD (Taoyuan, Taiwan) and contained 60% citral as determined by the HPLC method [17]. Citral (purity > 99%) was purchased from Sigma.

2.2. Animal studies

Experiment 1: We investigated the effect of LO and citral on drug-metabolizing enzymes and oxidative stress in rat livers. Male Sprague-Dawley rats (6 weeks) obtained from BioLASCO Taiwan (Ilan, Taiwan) were used. Rats were housed in plastic cages in a room kept at 23 ± 1°C and 60 ± 5% relative humidity with a 12-hour light-dark cycle. Food and drinking water were available *ad libitum*. The animals were fed a pelleted laboratory diet and administered 200 LO [200 mg/kg body weight (BW)], 400 LO (400 mg/kg BW), or citral (240 mg/kg body BW) by gavage for 2 weeks. The citral given to rats was equal to the citral content given to rats administered 400 LO. Corn oil was used as a vehicle for dissolving the LO and citral. The animals in all four groups were sacrificed by exsanguination via the abdominal aorta while under carbon dioxide (70:30, CO_2/O_2) anesthesia. Heparin was used as the anticoagulant and the plasma was separated from the blood by centrifugation (1750*g*) at 4°C for 20 min. The liver from each animal was immediately removed after exsanguination, weighed, and stored at −80°C. Microsome preparation and enzyme assays were performed within 2 weeks after liver collection. Drug-metabolizing enzymes, antioxidant systems, and oxidative stress in the liver were evaluated.

Experiment 2: To investigate the effect of LO and citral on the metabolism and toxicity of APAP, male Sprague-Dawley rats (6 weeks old) were randomly divided into four groups with eight rats in each group. The animals were fed a pelleted laboratory diet with vehicle (corn oil, Groups 1 and 2), LO (400 mg/kg BW LO, Group 3), or citral (240 mg/kg BW citral, Group 4) for 2 weeks. At the end of the experiment, food was withdrawn for 12 hours and a single 1000-mg/kg dose of APAP [18,19], as a solution in polyethylene glycol 400/water (50/50, v/v), was intraperitoneally injected into each animal in Groups 2, 3, and 4. At 12 hours after the APAP injection, the animals in all four groups were sacrificed. The plasma was separated from blood and the livers were removed immediately as described in Experiment 1. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were measured immediately by use of a commercial kit (Randox Laboratories, Antrum, UK).

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the China Medical University. The animals were maintained in accordance with the guide for the care and use of laboratory animals as translated by The Chinese-Taipei Society for Laboratory Animal Sciences, Taiwan.

2.3. Preparation of liver microsomes

Liver microsomes were separated by the method reported previously [20]. The frozen liver was thawed and then homogenized (1:4, w/v) in ice-cold 0.1M phosphate buffer (pH 7.4) containing 1mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 10,000*g* for 15 min at 4°C. The supernatants were then centrifuged at 105,000*g* for 60 min. The resulting microsomal pellets were suspended in a

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