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Original Article

Effects of aqueous extract of *Ruta graveolens* and its ingredients on cytochrome P450, uridine diphosphate (UDP)-glucuronosyltransferase, and reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H)-quinone oxidoreductase in mice

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ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form
29 January 2015
Accepted 24 March 2015
Available online 21 May 2015

Keywords:
cytochrome P450
furanocoumarins
Ruta graveolens

ABSTRACT

Ruta graveolens (the common rue) has been used for various therapeutic purposes, including relief of rheumatism and treatment of circulatory disorder. To elucidate the effects of rue on main drug-metabolizing enzymes, effects of an aqueous extract of the aerial part of rue and its ingredients on cytochrome P450 (P450/CYP), uridine diphosphate (UDP)-glucuronosyltransferase, and reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H):quinone oxidoreductase were studied in C57BL/6JNarl mice. Oral administration of rue extract to males increased hepatic Cyp1a and Cyp2b activities in a dose-dependent manner. Under a 7-day treatment regimen, rue extract (0.5 g/kg) induced hepatic Cyp1a and Cyp2b activities and protein levels in males and females. This treatment increased hepatic UDP-glucuronosyltransferase activity only in males. However, NAD(P)H:quinone oxidoreductase activity remained unchanged. Based on the contents of rutin and furanocoumarins

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http://dx.doi.org/10.1016/j.jfda.2015.03.005

rutin
UDP-glucuronosyltransferase

of mouse dose of rue extract, rutin increased hepatic Cyp1a activity and the mixture of furanocoumarins (F_{mix}) increased Cyp2b activities in males. The mixture of rutin and F_{mix} increased Cyp1a and Cyp2b activities. These results revealed that rutin and F_{mix} contributed at least in part to the P450 induction by rue.

1. Introduction

Drug-metabolizing enzymes play a crucial role in the biotransformation of xenobiotics, including drugs and chemical carcinogens, as well as physiological substances such as steroids. In Phase I metabolism, microsomal cytochrome P450 (P450/CYP)-dependent monooxygenase is the primary enzyme system that is responsible for oxidations. This monooxygenase system participates in the oxidation of a variety of endogenous and exogenous substrates, such as testosterone, methadone, and codeine [1]. P450 catalyzes the oxidations by transfer of two electrons through NADPH—P450 reductase (CPR), and the second electron can also be transferred by cytochrome b_5. Enzymes of the P450 superfamily exhibit broad substrate specificity and functional diversity. Therefore, selective substrates have been used as chemical probes for characterizing functional changes in individual P450 isoforms [1]. In the nomenclature of P450 isoforms, lowercase letters are used only for mouse P450, such as Cyp1a2 (http://drnelson.uthsc.edu/cytochromeP450.html). In Phase II metabolic reactions, uridine diphosphate (UDP)-glucuronosyltransferase (UGT)-catalyzed glucuronidation is quantitatively the most important conjugative reaction and appears to be the main metabolic pathway of a variety of drugs, such as acetaminophen. Reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H):quinone oxidoreductase (NQO) catalyzes further oxidation of quinone substrates, including the antineoplastic agent mitomycin C [2]. Functions of drug-metabolizing enzymes can be modulated by several natural products, such as psoralen derivatives [3–5]. Alterations of drug-metabolizing enzymes may result not only in herb—drug interaction, but also in changes in physiological functions of endogenous substrates or in carcinogenicity of xenobiotics.

Ruta graveolens L. (Yuin Siang), commonly known as rue, has been used in America, Asia, and Europe for the treatment of heart disease, wounds, and rheumatism [6,7]. In Taiwan, the aerial part of rue has been reputedly used as a folk medicine for the treatment of circulatory disorders. In Europe, this plant has also been used as an abortifacient and emmenagogue [6]. Aqueous extract of rue was found to reduce human sperm motility [8]. According to the 53-day spermatogenic cycle in rats, a study of 60-day treatment of rue was designed and the changes in the rue-treated group were compared to those in the vehicle control group [9]. Oral administration of rue aqueous extract at 0.5 g/kg/d to male adult rats for 60 days decreased their serum testosterone level, suppressed sperm motility and number of mounts, and prolonged ejaculatory latency. Types of chemical compounds identified in rue include coumarins, flavonoids, lignans, and quinoline alkaloids [10]. Furanocoumarins, such as 8-methoxypsoralen (MOP) and chalepensin (Fig. 1), represent one group of biologically active rue constituents and can be linked to the phototoxicity and antifertility of rue [10]. The flavonoid rutin (Fig. 1) has been identified as a water-soluble glycoside in rue [11]. In the evaluation of the uterotonic activities of rue fractionations, rutin was found in the active fraction and had the most potent uterotonic effect [11]. It has been suggested that furanocoumarins and rutin play important roles in the pharmacological/toxicological effects of rue.

In the modulation of drug-metabolizing enzymes, intraperitoneal administration of a single dose of 25 mg/kg 8-MOP to rats induced hepatic CYP2B after 24 hours and 48 hours [3]. Chalepensin activated the mouse constitutive androstane receptor (CAR) and induced Cyp2b9/10 in male C57BL/6JNarl mice after 1 week of treatment [4]. By contrast, some of the furanocoumarins in rue, such as 5-MOP, 8-MOP, and chalepensin, are known potent mechanism-based inhibitors of CYP2A and CYP3A *in vitro* and/or *in vivo* [5,12]. Rutin is catabolized by human intestinal/fecal bacteria to form deglycosylated products including leucocyanidin and quercetin [13]. Quercetin induced CYP1A1 expression in HepG2 cells,

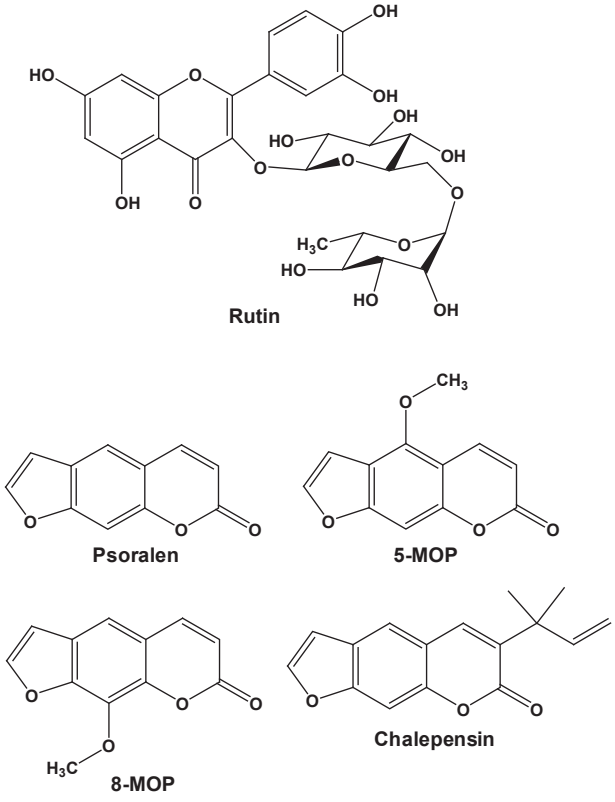

Fig. 1 — Structures of rutin and furanocoumarins identified in the rue aqueous extract. MOP = methoxypsoralen.