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Modulation of P450 enzymes by Cuban natural products rich in polyphenolic compounds in rat hepatocytes

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Abstract

This paper reports cytotoxic effects and changes in the P450 system after exposing rat hepatocytes to four polyphenol-rich products widely used in Cuban traditional medicine (*Mangifera indica* L. (MSBE), *Thalassia testudinum* (Tt), *Erythroxylum minutifolium* and *confusum* extracts). Effects of mangiferin, the main polyphenol in MSBE, were also evaluated. Cytotoxicity was assayed by the MTT test after exposure of cells to the products (50–1000 μg/mL) for 24 or 72 h. The results showed that 500 μg/mL MSBE was moderately cytotoxic after 72 h, while mangiferin was not. Marked reductions in cell viability were produced by *Erythroxylum* extracts at concentrations ≥200 μg/mL, whereas only moderate effects were induced by 1000 μg/mL Tt. Seven specific P450 activities were evaluated after 48 h exposure of cells to the products. MSBE reduced phenacetin *O*-deethylation (POD; CYP1A2) activity in a concentration-dependent manner (IC_{50} = 190 μg/mL). No decreases were observed in other activities. In contrast, mangiferin produced reductions in five P450 activities: IC_{50} values of 132, 194, >200, 151 and 137 μg/ml for POD (CYP1A2), midazolam 1′-hydroxylation (M1OH; CYP3A1), diclofenac 4′-hydroxylation (D4OH; CYP2C6), *S*-mephenytoin 4′-hydroxylation (SM4OH), and chlorzoxazone 6-hydroxyaltion (C6OH; CYP2E1), respectively. *E. minutifolium*, *E. confusum* and Tt extracts produced small reductions in SM4OH and C6OH activities, but no significant changes were noted in the other P450 activities. On the other hand, all the products increased the benzyloxyresorufin *O*-debenzylation (BROD; CYP2B1) activity, with MSBE, mangiferin or *E. minutifolium* showing the highest effects (about 2-fold over control). Our results showed *in vitro* effects of these natural products on P450 systems, possibly leading to potential metabolic-based interactions.

Keywords: Cuban herbal products; Rat hepatocytes; Cytochrome P450; Polyphenols

1. Introduction

Herbal extracts have been used as traditional remedies for the treatment of diseases for almost 2000 years and are being increasingly used worldwide. Herbal remedies

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are, however, not risk-free since herb–drug interactions and other safety issues have been reported. In fact, patients often combine prescription medications with herbal and dietary substances, thus affecting the disposition of conventional pharmaceuticals through the inhibition/induction of human cytochrome P-450 (P450) enzymes.

It is well known that P450 is the most important phase I drug-metabolizing enzyme system responsible for the metabolism of a variety of xenobiotics, including drugs and endogenous substances such as steroids [1–3]. Approximately, 70% of human liver P450 is accounted for by CYP1A2, 2A6, 2B6, 2Cs, 2D6, 2E1 and 3A enzymes [4]. Among them, CYP3A4, 2D6, and 2Cs are highly responsible for the metabolism of most drugs in current use, whereas other P450s (CYP1A2, 2A6, 2B6, 2E1) are greatly involved in the metabolic detoxication/bioactivation of other xenobiotics [2,4,5].

Several examples have been reported in the literature that show herb–drug interactions after the co-administration of herb constituent(s) along with pharmaceutical drugs, which may lead to adverse drug interactions. These interactions occur by modulating the P450 system, including the induction or inhibition of specific P450 enzymes and the metabolic clearance of the drug [6,7]. A typical example is St. John's wort, widely used for depressive disorders, which is a potent inducer of CYP3A4. Yet it also contains ingredients that inhibit CYP1A2, 2C9, 2C19 and 2D6 [8]. Another example is the Echinacea purpurea extract of well-established therapeutic usage in the protection of upper respiratory tract infections, and which has been described as a potent inhibitor of CYP3A4, 2D6 and 2C19 [9,10]. Finally, naturally occurring flavonoids, a group of phytochemicals displaying a wide range of biological activities, have also been shown to modulate the P450 system [11]. Therefore, special attention should be paid to the potential effects of herbal extracts, or their components, on P450 enzymes, and to the pharmaco-toxicological consequences when they are co-administered with other drugs.

Herbal medicines have been used for many years in Cuba for the treatment of several diseases. In recent years, there is an increasing consumption of herbal extracts (*Mangifera indica* L., *Thalassia testudinum*, *Erythroxylum minutifolium* and *E. confusum*) that are often administered in combination with conventional therapeutic drugs. These extracts are used in the treatment of different pathologies, such as the prevention of age-associated oxidative stress in the elderly and to improve patient welfare. Formulations of herbal extracts are used by patients suffering pain, inflammation or burns, and in the treatment of immunopathological disorders, including bronchial asthma, atopic dermatitis and other allergic diseases.

Mango (*M. indica* L., Anarcadeaceae) stem bark aqueous extract (MSBE) has been developed in Cuba as a new natural product with a defined composition, whose brand name is Vimang®. Between 2001 and 2007, from 60 to 900 kg of Vimang has been sold annually as a nutritional supplement to improve the welfare of those patients suffering different types of pain and inflammation [12]. A topical preparation of the *T. testudinum* marine plant is registered and widely used in Cuba to protect burns and as an anti-aging cream [13]. At present, a new oral formulation has been developed as an anti-inflammatory and hepatoprotective supplement, and as an aid for liver diseases. Finally, *Erythroxylum* extracts have been used as topical unguents for bacterial and/or viral infections of the skin, and decoctions are orally consumed by people for renal and respiratory affections.

Chemical analysis of these extracts revealed that polyphenols are the main constituents responsible for their beneficial properties [12–16]. Plant polyphenols are an important group of chemicals with a recognized capacity to modulate P450 enzymes [17]. Thus, the aim of the present study was to investigate the potential inhibitory or inductive effects of these herbal extracts on the P450 system. The effects of mangiferin, the main polyphenolic component of *M. indica* L., were also analyzed. First, the cytotoxic effects of the test products on cultured rat hepatocytes were determined and then their effects on seven P450 activities were studied.

2. Materials and methods

2.1. Chemicals

Collagenase and β-glucorinadase/arylsulfatase were obtained from Roche (Barcelona, Spain). Ham's F-12 and Lebovitz L-15 medium and calf serum were acquired from Gibco (Madrid, Spain). Bovine serum albumin, benzoxyresorufin, resorufin, diclofenac, bufuralol, 1′-hydroxybufuralol, chlorzoxazone, *S*-mephenytoin and 4′-hydroxymephenytoin were purchased from Sigma Chemical Co. (Madrid, Spain). 4′-Hydroxydiclofenac, 6-hydroxychlorzoxazone, midazolam and 1′-hydroxymidazolam were supplied by Ultrafine (Manchester, UK). All other reagents used were of the purest grade available.