Interactions of Polyphenols with the P450 System: Possible Implications on Human Therapeutics

I. Rodeiro[1], M.T. Donato[2, 4], A. Lahoz[3], G. Garrido[1], R. Delgado[1] and M.J. Gómez-Lechón[4,*]

[1]Laboratorio de Farmacología, Departamento de Investigaciones Biomédicas, Centro de Química Farmacéutica, Ciudad de la Habana, Cuba; [2]Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Valencia, Spain; [3]Unidad Mixta Advancell-Hospital La Fe. Avda Campanar 21, 46009-Valencia. Spain; [4]Unidad de Hepatología Experimental. Centro de Investigación, Hospital La Fe, Avda Campanar 21, 46009-Valencia, Spain

Abstract: Polyphenols are a family of natural compounds with many biological properties. This review focuses on their potential interaction on the cytochrome P450 system. Effects of phenolic acids, anthocyanins, stilbenes, catechins and other flavonoids on the drug metabolising function are revised. Their daily intake and presence in herbal medicines justify the study of potential drug-interaction to prevent undesirable clinical consequences.

Key Words: Antioxidants, flavonoids, polyphenols, cytochrome P450, herbal medicines.

INTRODUCTION

Polyphenols are a large group of natural compounds that includes flavonoids, anthocyanins, and tannins, which are distributed throughout food sources and ingested daily in significant amounts as part of diets. These compounds are present in many fruit, vegetables and beverages, such as red wine and tea, and their daily intake ranges from 0.2-1g worldwide. It has been proposed that polyphenols are beneficial agents for several pathologies. Potent antioxidant effects of these molecules have been reported, which in turn depend on both the free radical scavenging capacity and the iron chelating activity, among other biological properties [1-5]. Polyphenols are a major source of antioxidants consumed by humans and are serious candidates to explain the protective effects of fruit and vegetables against oxidative damage diseases (coronary heart disease, stroke, and cancers). Antiviral, antibacterial, anti-inflammatory, neuroprotective and anti-carcinogenic action, as well as the ability to modulate certain signalling pathways such as nuclear factor-kappa B activation, have also been attributed to natural polyphenols [6-11]. Therefore, plant polyphenols are a versatile group of phytochemicals with many potentially beneficial activities in terms of disease prevention.

Due to the increasing use of herbal medicines worldwide, a growing interest is now being shown to the therapeutic properties of these natural products as well as to the potential clinical implications of their consumption [12-14]. One of the reasons for their growing popularity is the belief that they are natural and therefore 'safe'. Unfortunately, the quality of the majority of them remains essentially uncontrolled because they are not generally categorized as medicines. Herbal products are not tested with the scientific rigor required of conventional drugs. Besides, they are not subject to the approval process of drug regulatory agencies such as the Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA). As a consequence, herbal medicines and plant foods generally contain a vast array of complex constituents, which can potentially interact with other therapeutic agents.

Drug interactions during therapeutics management can range from clinically irrelevant to fatal, and may lead to significant toxicity or treatment failures [13, 15]. Of the pharmacokinetic factors that control drug action, the metabolism rate is one of the most important. Hence, metabolism-based interactions can be of great clinical significance. Such interactions occur when a certain component alters the activity of a drug-metabolizing enzyme, leading to a modulation of the pharmacokinetics of drugs metabolized by the enzyme. A variety of interactions of herbs or foods (grapefruit juice, red wine, St John's wort) with drugs have been documented to date, most of them involving interactions in one or multiple cytochrome P450s, the major drug-metabolizing enzymes [16]. The widespread use of herbal remedies and the incomplete knowledge about their metabolic disposition has led to a heightened awareness of physicians and researchers with regard to the potential underlying herb-drug interactions.

The present study focuses on the potential interaction of polyphenols on the drug-metabolizing function, and particularly on oxidative reactions catalyzed by the P450 system. The effects of polyphenolic compounds on the expression and/or activity of P450 enzymes have been reviewed. The presence of these molecules on many herbal medicines and foods, and the increasing popularity of plant-derived extracts and preparations as alternative medicines recommend an extensive knowledge of the possible implications on the human therapy of the modulation of the P450 function by polyphenols. Special attention is paid to relevant herb-drug interaction information, which can occur when a herbal supplement is coadministered with a drug primarily cleared by a single metabolic route.

CYTOCHROME P450 SYSTEM

Biotransformation is a process by which cells modify the xenobiotics that they enter into contact with, whose ultimate

*Address correspondence to this author at Unidad de Hepatología Experimental, Centro de Investigación, Hospital La Fe, Avda Campanar 21, 46009-Valencia, Spain; Tel: +34 96 1973048; Fax: +34 96 1973018;
E-mail: gomez_mjo@gva.es

1389-5575/08 $55.00+.00

goal is to facilitate their elimination. To achieve this, lipophillic compounds, which would otherwise accumulate in intracellular lipids, are rendered more hydrophilic by a set of broad-specificity enzymes capable of either introducing new functional groups (Phase I reactions) or conjugating with hydrophilic endogenous molecules to increase water solubility (Phase II reactions). The formation of water-soluble metabolites not only enhances drug elimination, but also leads to molecules that are generally pharmacologically inactive and relatively non-toxic [17].

Phase I reactions include oxidative, reductive and hydrolytic biostransformations which primarily serve to increase the hydrophilicity and to facilitate the excretion of xenobiotics. This occurs by incorporating a new polar functional group into the parent molecule (OH, COOH, NH_2, SH) or by unveiling/modifying existing functionalities (i.e. oxidative O-, N-, and S-dealkylations, reduction of ketone and aldehide to alcohols, reduction of azo and nitro compounds, hydrolysis of ester and amides). Phase II comprises different types of reactions, including the conjugation of the parent molecule (or its phase I metabolites) with endogenous molecules (i.e. glucuronic acid, glutathione, sulphate, etc.). Conjugates tend to be much more water-soluble, more easily excreted in the bile and/or urine and usually a lot less active (less toxic) than non-conjugated compounds [17].

Cytochrome P450 (P450) enzymes, as well as flavin monooxygenases, are major role players in the phase I oxidative metabolism of a wide range of structurally diverse xenobiotics (drugs, environmental pollutants, carcinogens, dietary components, chemicals) and in the metabolism/biosynthesis of endobiotics (steroids, fatty acids, prostaglandins, bile acids) [18, 19]. Both phase I enzymes oxidize substrates make use of one of the two atoms of oxygen (monoxygenases), while the other is reduced to H_2O by NADPH. P450s are a superfamily of enzymes bound to membranes of the endoplasmic reticulum and are associated with other proteins (cytochrome P450 reductase and cytochrome b5), which provide the electrons required for the monooxygenase reaction. P450 enzymes are grouped into families and subfamilies according to their amino acid sequence homology [for the CYP nomenclature, see 20] (Fig. **1**). Three main P450 gene families, CYP1, CYP2 and CYP3, are responsible for xenobiotic metabolism. P450 is predominantly expressed in the liver although it is also present in extrahepatic tissues, such as the lung, kidney, grastrointestinal tract, skin, brain and placenta [21-23]. The existence of multiple P450s and the catalytic versatility of the P450 system are primarily responsible for the efficient hepatic oxidation of drugs. In the human liver, xenobiotics are predominantly biotransformed by a limited subset of P450 enzymes, namely CYP1A2, 2A6, 2B6, 2Cs (principally CYP2C9 and 2C19), 2D6, 2E1, 3A4 and 3A5 [24] (Fig. **2**). CYP3A4 is the most abundantly expressed P450, representing around 30-40% of the total P450 protein in adult human liver [25]. Together with CYP2C9 and 2D6, they account for the metabolism of most currently used pharmaceuticals (Fig. **2**).

Variability of drug metabolism rates is a consistent observation in human populations. Inter-individual differences in catalytic activities are attributed to genetic polymorphisms and to the role of non-genetic factors (i.e., hormonal status, age, diet, smoking status, ethanol intake, exposure to chemicals) in the control of the P450 expression. It is well documented that P450 enzymes are polymorphically expressed [19, 26]. Mutations of P450 genes result in allelic variants causing defective, qualitatively altered, diminished or enhanced rates of drug metabolism. As a consequence, polymorphisms can lead to qualitative and/or quantitative alterations in the metabolism of drugs, and could be responsible for the development of a number of unexpected adverse drug reactions and host-specific susceptibility to drugs or other chemicals.

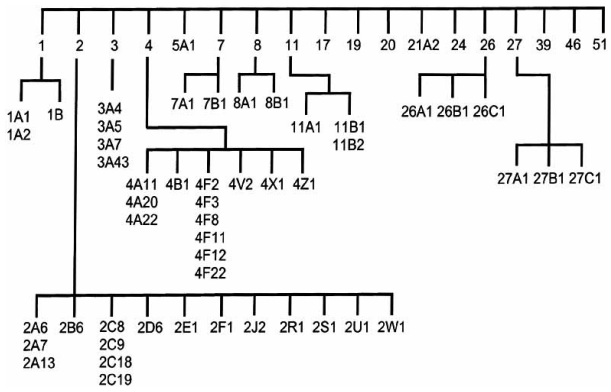

Fig. (1). Human P450 enzymes.

A characteristic of many P450 enzymes is their inducibility by xenobiotics and, in particular, by drugs. Upon repeated administration, certain drugs can increase their own metabolism (or that of other therapeutic agents) by altering the expression of P450s and other drug metabolizing enzymes. Most human drug-metabolizing P450s are either polymorphic or inducible, which accounts for the large inter-individual variability found in humans [27]. Moreover, as xenobiotics are metabolized by a limited number of enzymes, they can compete among themselves as substrates/inhibitors for P450 enzymes. Hence, the presence of one chemical can interfere with the metabolism of another. Inhibition of a metabolically relevant P450 by a particular compound can result in elevations in the plasma/tissue concentration of other coadministered drugs. Changes in pharmacokinetic properties often result in an inadequate or variable clinical response of the drug that can compromise its therapeutic usage. Given the importance that the P450 phenotype has in pharmacokinetics, pharmacodynamics and the potential toxicity of a given drug, the identification of potential factors altering the enzyme expression and/or function is of relevance. Such phenomena are at the root of potential drug-drug interactions.

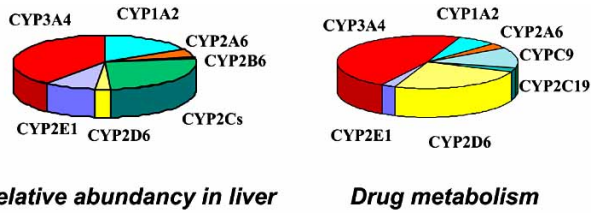

Fig. (2). Human P450 enzymes involved in hepatic drug metabolism.

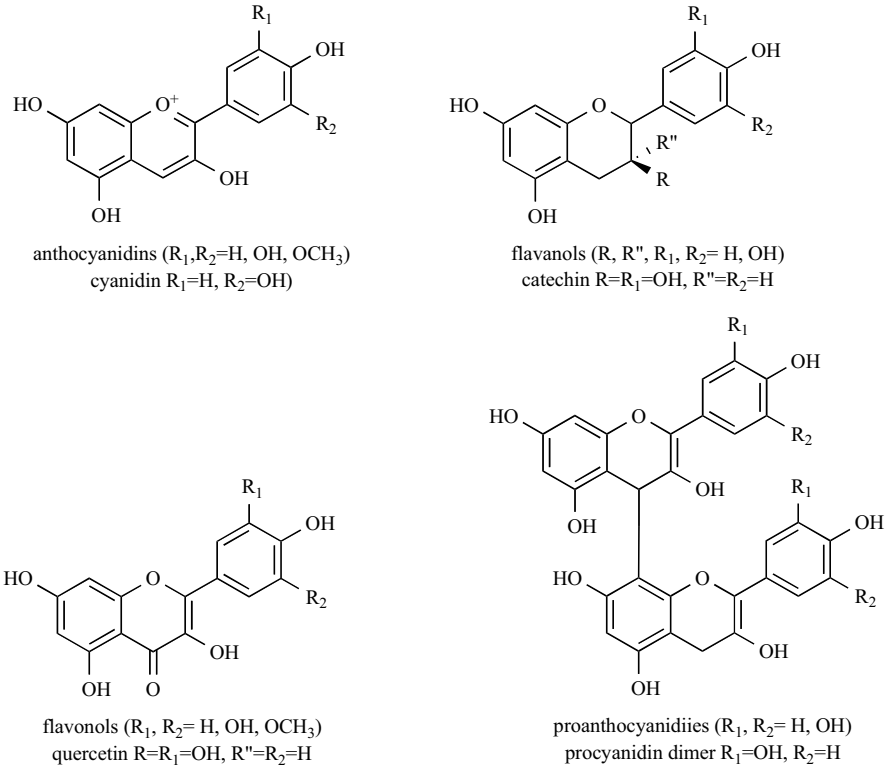

Fig. (3). Chemical structure of the main classes of flavonoids polyphenols.

CHEMICAL STRUCTURE AND BIOAVAILABILITY OF POLYPHENOLS

Polyphenols represent a wide variety of compounds which are categorized into different classes according to the chemical nature of their carbon skeleton. However, it is impossible to know the chemical nature of all the phenolic compounds that we intake as part of our diet. Polyphenols are often classified into two main groups, flavonoids (Fig. **3**) and non-flavonoids which comprise phenolic acids, and the less common stilbenes and lignans (Fig. **4**) [28].

Flavonoids are the most abundant polyphenols subclass present in our diets, characterized as containing two aromatic rings or more, each bearing at least one hydroxyl group and connected with a carbon bridge [29]. Flavonoids are divided

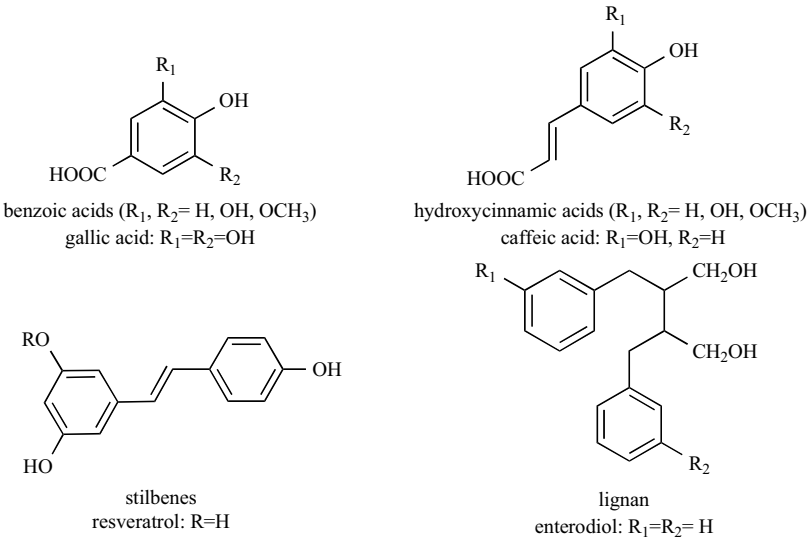

Fig. (4). Chemical structures of the main classes of nonflavonoid polyphenols.

into several classes according to the degree of oxidation of the oxygen heterocycle: flavones, flavans, flavonols, isoflavones, anthocyanins, flavanols, proanthoyanidins and flavanones [30]. Many flavonoids are polymerized into larger molecules; such polymers are called tannins. Condensed tannins, or proanthocyanidins, consist of monomeric units of flavans linked through carbon-carbon and ether linkages.

Phenolic acids are abundant in plant extracts. The most frequently encountered are caffeic and ferulic. Lignans, another subclass of biologically active polyphenols, have a four-carbon bridge between the two aromatic rings leading to many different structures. The last class of polyphenols is the stilbenes in which the two aromatic rings are linked by a two-atom carbon linker containing a double bond.

Plant extracts comprise a great variety of polyphenols, such as flavonoids, simple phenolic acids (benzoic acids and hydroxylcinnamic acids), stilbenes and also complex molecules which derive from them, such as proanthocyanidines [31-38] (Figs. **3** and **4**). Huge numbers of flavonoids have been identified in plants and are mainly due to the occurrence of many numerous substitution patterns in which primary substituents (e.g. hydroxyl, methoxyl or glycosyl groups) can be substituted (acylated). Furthermore, the composition of plant polyphenols is highly variable qualitatively and quantitatively [39].

Polyphenols are abundant nutrients in our diet. However, their bioavailability varies widely among them, and among dietary sources for some compounds. Many studies have been published to investigate the kinetics and the extent of polyphenols absorption by measuring plasma concentrations and/or urinary excretion after the intake of a dose of a plant extract [40, 41]. Bioavailability of polyphenols has been comparatively reviewed [41]. As shown, the most striking feature of that survey was that gallic acid is far better absorbed than other polyphenols. On the other hand, proanthacyanidines and anthocyanidines are poorly absorbed. The most well absorbed polyphenols in humans are isoflavones and gallic acid followed by cathecins, flavonones and quercetin glucosides. Most polyphenols glucosides are deglycosylated by β-glycosidases in the small intestine; this step is a requisite for the absorption of many of these polyphenols [42].

EFFECTS OF PLANT-POLYPHENOLS ON THE CYTOCHROME P450 SYSTEM

To date, several studies have been performed to study the effects of plant-polyphenolic on P450 isoenzymes (Table **1**). Polyphenols can modulate cytochromes in two manners, modulating the expression, or/and the activity of the enzyme.

Flavonoids

Flavonoids can directly modulate the activities of various P450 [43-50] and are identified as substrates of the P450 system [45, 51]. In the liver, flavonoids are hydroxylated and/or *o*-demethylated by various P450s and thereafter conjugated by phase II enzymes for their elimination [12].

Among them, catechins of green tea are the most studied flavonoids [10, 52]. Muto *et al*. [53], demonstrated that epigallocatechin gallate, epicatechin gallate, epigallocatechin and epicatechin inhibit the *in vitro* activity of CYP1A1/2 and CYP3A4. Moreover, a general inhibition of several P450s (CYP2A6, CYP2C19, CYP2E1) by epigallocatechin gallate has been observed, indicating a non-specific inhibitory effect [53, 54]. The activation of benzo(a)pyrene, 2-amino-1-methyl-6-phenylimidazole-(4,5-b)pyridine and aflatoxin B-1 by recombinant CYP1A1/2 and CYP3A4, respectively, was also inhibited by these molecules [53]. These effects have been associated with the chemopreventive properties of catechins observed in rodents. The potential interactions of green tea catechins on the metabolic activation of irinotecan, a prodrug directed to the treatment of metastatic colorectal cancer have been recently suggested [55]. Other studies indicated that a modulation of the human CYP1A expression by green tea extracts can be attributed entirely to the combination of the four tea catechins present in the extract more than one component *per se* [54]. Meanwhile, there are also experimental evidences that show green tea as an inducer of CYP1A and CYP2B activities and as the expression of CYP1A2 in rats [56, 57]. In this sense, this appositive modulating effect of herbal tea and catechins on CYP1A has been attributed to the presence of caffeine in herbal teas, a potent inducer of CYP1A2. The differences in catechin concentrations and exposure times may also contribute to these observed effects [12, 58]. Recently, a heteroactivation of CYP1A1 by teas and tea polyphenols was observed, which supports the hypothesis that this mechanism of CYP1A1 activation may be part of the explanation for the lack of epidemiological support for cancer prevention observed by tea [59].

Flavone is an inhibitor of enzymes of the CYP1A subfamily and shows a higher affinity to CYP1A2 than to CYP1A1. Several hydroxylated flavone derivates (3-hydroxy-, 5-hydroxy-, 7-hydroxy, and 3,7-hydroxyflavone) have been identified as potent inhibitors of CYP1A1/2 enzymes. 7-Hydroxyflavone is a competitive inhibitor for CYP1A1 and is 6-fold more selective for CYP1A1 than for CYP1A2. In contrast, galangin (3,5,7-tridroxyflavone) shows a mixed-type inhibition with a higher inhibitory potency toward CYP1A2 activity [60]. Likewise, 7,8-benzoflavone is an inhibitor of human CYP1A2, but an activator of CYP3A4 [61].

A comparison of inhibitory potency of different flavonoids revealed that myricetin, quercetin and kaempferol (flavonols) and apigenin (a flavone), strongly inhibited diolepoxide formation (catalyzed by CYP1A1), whereas flavanones (naringenin) and flavonoid glycosides (rutin) exhibited a slight inhibitory capacity toward diolepoxide formation (IC_{50} values >100 μM). Thus, the inhibitory effects of flavonoids on CYP1A1 activity depend on both the structure of the inhibitor and the type of reaction (the structure of substrate) studied [62, 63].

Biochanin A and formononetin, predominant isoflavones in red clover, are converted to genistein and daidzein in human microsomes, respectively. Daidzein is a competitive inhibitor of CYP1B1 and genistein exhibited mixed inhibition. Extrahepatic human cytochromes P450 1A1 and 1B1 are also inhibited by the metabolism of isoflavones present in red clover [64]. Other flavonoids, such as equol or hop,

Table 1. Potential Metabolic-Based Interactions Produced by Polyphenols

Polyphenol	Type of interaction	CYP enzyme	References
Flavonoids			
7,8 benzoflavone	Inhibition Induction	CYP1A2 CYP3A4	[61]
Apigenin	Inhibition	CYP1A1	[63]
β-naphtoflavone	Induction	CYP1A1/2, CYP2B1/2	[67,75]
Butein (3,4,V,4V-tetrahydroxychalcone)	Inhibition	CYP19	[33]
Catechins of Green tea		CYP1A1/2, CYP3A4	[61]
Epigallocatechin gallate	Inhibition	CYP2A6, CYP2C19, CYP2E1	[53, 67]
Daidzein	Inhibition	CYP1B1	[64]
Diosmetin	Induction	CYP1A1/2, CYP2B1/2	[67,75]
Diosmin and its aglycone	Induction	CYP1A1/2, CYP2B1/2	[67,75]
Flavanone	Induction Inhibition	CYP2B1/2 CYP3A4	[77] [68]
Flavone, hydroxyflavones as galagin	Inhibition	CYP1A1/2, CYP19	[125,143]
Genistein	Inhibition	CYP1B1	[64]
Kaempferol	Inhibition	CYP1A1, CYP3A4	[63,67,68]
Methoxylated flavonoids (5,7-dimethoxyflavone; 30,40-dimethoxyflavone)	Inhibition	CYP1A1/1B1	[34,69]
Myricetin	Inhibition	CYP1A1	[63]
Quercetin	Inhibition	CYP1A1, CYP3A4, CYP2C8	[15,62,66]
Tangeretin	Induction	CYP1A1/2, CYP2B1/2	[67,73]
Non flavonoides			
Caffeic acid	Inhibition	CYP1A1/2,CYP2B	[92]
Ellagic acid	Inhibition	CYP2E1	[85]
Gallic acid	Inhibition	CYP3A	[87]
Resveratrol	Inhibition	CYP1A1/2, CYP2B, CYP3A4, CYP1B1	[43,63,71,93-95]
Resveratrol analogues (piceid, resveratroside, methoxystilbenes)	Inhibition	CYP3A4	[95]
Viniferin	Inhibition	CYP1A1, CYP1B1, CYP2B6, CYP2A6, CYP2E1, CYP3A4, CYP4A	[94]

prenylflavanones and prenylchalcones do not modulate P450 enzymes [65].

Reduced CYP1A function produced by certain flavonoids occurs as a consequence of a decrease in enzyme levels rather than a direct interaction of the chemical with the enzyme. Quercetin binds as an antagonist to the Ah receptor with the consequent inhibition of benzo(a)pyrene-induced CYP1A1 mRNA transcription and protein expression, thus resulting in a reduction of benzo(a)pyrene-DNA adduct formation [62,66]. Kaempferol prevents CYP1A1 gene transcription induced by the prototypical Ah receptor ligand, TCDD and it is an inhibitor of CYP3A4 [67,68]. Methoxylated dietary flavonoids, e.g. 5,7-dimethoxyflavone and 5,7-dimethoxyflavone, directly inhibit the CYP1A/1B1 function and/or its protein expression [34]. 5,7-Dimethoxyflavone is a potent inhibitor of benzo(a)pyrene-induced DNA binding, the CYP1A1 protein expression, and of the activity in Hep G2 cells [69].

The inhibition of the gene expression of the CYP1 subfamily by flavonoids by blocking the Ah receptor has been associated with an important role in their cancer chemopreventive properties. Several studies support the hypothesis that they may be involved in preventing a malignant transformation by reducing the formation of carcinogens through the inhibition of CYP1A1/2 [66, 69-72]. In general, the results of the studies indicated that flavonoids have a high potency and selectivity for the inhibition of CYP1A isoforms. This modulation on CYP1A has also been associated with the fact that the dietary exposure to flavonoids may contribute to the inter-individual variation in pharmacokinetics and to pharmacological responses observed for drugs such as phenacetin, caffeine, theophylline and propanolol, substrates for CYP1A2 [73,74] as well as for drugs which are substrates of other P450s.

Although most described inhibitory effects of flavonoids on the P450 system concern CYP1A and CYP1B enzymes, the inhibition of other drug/xenobitic-metabolizing P450s, such as CYP2A6, 2C18, 2C19, 2E1, and 3A4, or CYP19 (involved in the metabolism of endogenous compounds), has also been reported (Table **1**). By way of example, quercetin increased the oral bioavailability of paclitaxel in a dose-dependent manner [15]. This flavonoid was identified as an inhibitor of CYP3A4 and 2C8, enzymes responsible for the metabolism of paclitaxel. The terminal elimination half-life of the drug was prolonged with quercetin, suggesting that the inhibition of P450-dependent metabolism resulted in a decreased clearance, although an inhibition of Pgp-mediated efflux was also observed [15].

Regulation of the P450 function by flavonoids is not limited to the inhibition of catalytic activities or to the down-regulation of the enzyme expression, as already described; i.e., P450 induction in response to certain flavonoids (Table **1**). Tangeretin, diosmin and its aglycone form, diosmetin and synthetic β-naphthoflavone stimulate the expression of CYP1A1/2 and CYP2B1/2 [57, 67, 75, 76]. Flavanone appears to be a specific inducer of CYP2B1/2 enzyme and an inhibitor of CYP3A4 [68, 77]. The mechanism described for the induction of P450 by flavonoids is apparently involved in the direct stimulation of a gene expression *via* a specific receptor and/or P450 protein or mRNA stabilization [78,79]. *In vitro* studies have shown an increased CYP1A1 expression after exposure to galangin and quercetin in the absence of other enzyme inducers. This contrasts with the CYP1A1 inhibition observed in the presence of inducers, such as the carcinogen dimethylbenz[a]anthracene [80]. Apparently, flavonoids could act as AhR inducers in the absence of a carcinogen, but they may act as antagonists in its presence. Consequently, these results suggest that whereas a co-exposure to flavonoids and carcinogens may be cancer preventive, flavonoid pre-treatment may prime the P450 system to metabolically activate pre-carcinogens [81]. The *in vivo* significance of such a P450 induction by flavonoids remains to be demonstrated. Extrapolating *in vitro* results to an *in vivo* situation requires extreme care. However, experimental studies provide a reasonable starting point for the assessment of potential clinical interactions [14].

In summary, some flavonoids are potent inhibitors of P450 enzymes, but others can act as inducers. The inhibition or activation of human P450 by flavonoids largely depends on their structures, concentrations and assay conditions. Several studies have been conducted to elucidate the structural features of flavonoids that are responsible for modulating P450 activities. The comparative study of the effects of green tea polyphenols and epicatechin derivatives on P450 activities suggested that inhibitory effects on the molecule may be due to both the hydroxyl and galloyl groups [82]. An analysis of a series of hydroxyl-substituted flavonoids on human CYP1 family activities showed that 3- and 5-hydroxylation significantly increase the inhibition of CYP1A2 activity, and conversely, 7-hydroxylation markedly decreases it [60, 83]. In contrast, the binding environment of the CYP1A1 active site has a preference for the 7-hydroxyl substituent. In addition to the substituent position, its nature also influences the inhibitory potential. In fact, large hydrophobic groups at position 7 elicit a higher affinity for CYP1A2 than the hydrophilic hydroxyl substituent (i.e. 7,8-benzoflavone *vs.* 7-hydroxyflavone), which contributes to an increase in enzyme inhibition. In contrast, no apparent correlation between the presence of 5- and/or 7-hydroxyl groups and the degree of CYP1B1 inhibition was found [83].

Non Flavonoids Polyphenols

Ellagic acid is a naturally occurring plant polyphenol showing broad biological activities [84]. Treatment of rats with oral doses of this compound resulted in a decrease of the total hepatic P450 content, cytochrome P450 reductase and CYP2E activities. However, no changes with CYP1A1, 2B1 and 3A activities were observed [85].

Gallic acid is mainly found in wine and tea. The exposure of repeated oral doses of gallic acid to mice resulted in an increase of the total hepatic P450 content and not changes were found in CYP1A [86]. Further studies showed gallic acid is a reversible inhibitor of the activity of CYP3A in liver human microsomes, and that the removal of gallic acid-derived products from incubation restored CYP3A activity [87]. In contrast, the activities of CYP1A and CYP2E were refractory to an inhibition by the polyphenol. Effects of gallic acid and its esters on drug-metabolizing activities in the activation of pre-mutagens have also been reported [88].

Resveratrol, a stilbene found in red wine, grapes and peanuts, has many biological effects, including antioxidant, anticancer, anti-inflammatory and anticoagulant activities [4, 89, 90, 91]. Resveratrol inhibits several CYP1A, 1B1, 2B, and 3A4 dependent activities [71, 92-94], and exhibits a slight inhibitory capacity toward diolepoxide formation [63]. A mechanism-based inactivation of CYP3A4 was suggested where resveratrol should also act as a substrate and produce an irreversible inhibition [94,95]. Several resveratrol derivatives or related compounds show P450 inhibitory properties. Viniferin, a dimmer of resveratrol, inhibited CYP1A1, 1A2, 1B1, 2A6, 2B6, 2E1, 3A4 and 4A activities in human liver microsomes. Viniferin is a more potent inhibitor than resveratrol for all the P450 activities tested. Like resveratrol, a mixed-type inhibition was observed for all the P450, except for CYP2E1 (non-competitive) [96]. Other resveratrol analogs (piceid, resveratroloside, 5,40-dihydroxy-3-O-methoxystilbene, and 5,3-dihydroxy-40-O-methoxystilbene) also inhibited CYP3A4 activity [95]. A comparative analysis of resveratrol and these four analogs was done to characterize

the structure-activity relationship of CYP3A4 and polyphenolic substrates. Methoxy-stilbenes showed lower IC_{50} values than the other compounds, suggesting that lipophilicity determines the CYP3A4 inhibition capacity of polyphenols rather than the number or positions of free hydroxyls [95]. The substitution of resveratrol hydroxyl groups produces chemically related structures with variable biological activities. It is generally recognized that the hydroxyl at the 4' position is the major functional group in scavenging free radicals and polyphenolxidase-dependent oxidation. However, the 4'methoxy analog was as good a substrate/inhibitor of CYP3A4 as the 3-methoxy one, which suggests that the inhibition mechanism is not affected by reducing either the potential or configuration of hydroxylgroups [95].

Lignans are metabolized in the gut to produce the phytoestrogens enterolactone and enterodiol. Genetic susceptibility to breast cancer has been associated with CYP17 polymorphism. Studies performed by McCann *et al*. [97] demonstrated that women with a higher intake of dietary lignans were less likely to develop breast cancer. These results suggested that the CYP17 genotype may be important in modifying the effect of exogenous estrogens on the risk of breast cancer, particularly for pre-menopausal women, and lignans consumption could modulate it.

HERBAL MEDICINES AND P450 ENZYMES.

Alternative therapies, such as herbal or natural products, are increasingly used around the world. Pharmacological properties of herbal remedies have been largely recognized in many countries as basic elements of traditional medicine. Recently, the popularity of such preparations in Western countries has notably increased and many have been incorporated as part of unconventional therapy [98-100]. Herbal medicines are a mixture of more than one active ingredient, and in many cases, polyphenols are a significant part of them. Polyphenols are involved in many pharmacological properties attributed to herbs and it has been recognized that they are also involved in the modulation of P450 enzymes by herbs. The level of use combined and the increasing complexity of the composition of herbal remedies have led to a heightened awareness of physicians and researchers about the potential drug-herbal interactions. The relevance of such interactions has not been widely addressed, although a number of case reports document the potential interactions and clinical impact derived from the intake of these natural products [for a review, see 14, 81,101]. In this section, reports on the effects of the most commonly used herbal products are briefly summarized.

St. John's wort *(Hypericum perforatum)* is the most studied plant in the world for its pharmacological properties. Among the chemicals identified in the extract, hypericim and hyperforin are believed to be active ingredients, but it also contains flavonols, flavonol glycosides, biflavones, naphthadianthrones, acylphloroglucinols and phenylpropanes [102, 103]. Because of its use in the treatment of major depression, it is quite probably used concomitantly with conventional drugs, including synthetic antidepressants (i.e., serotonin reuptake inhibitors).

Studies performed *in vitro* demonstrated that St. John's wort extract is a potent inducer of CYP3A4 and 2B6 [104]. Hyperforin showed an induction of the CYPA3A4 expression on primary human hepatocytes. Hyperforin is a ligand for the pregnane X receptor, which has been demonstrated as an orphan nuclear receptor regulating expression of CYP3A4 and 2B6 [105]. Meanwhile, I3-II8-biapigeni and quercetin are also involved in the inhibitory effects observed on P450s. Reports of Obach [106,107] showed that St John´s wort extract presented an inhibition on the activities of CYP1A2, 2C9, 2C19, 2D6 and 3A4. Studies performed in rodents demonstrated that this extract has effects on different P450s *in vivo* indicating that the dosing regimen and exposure time may modify the effects on P450s activities and expressions [108, 109].

Clinical studies have shown an induction of intestinal and hepatic CYP3A4 and other possible P450 enzymes after a two-week treatment with this product [110,111]. Different clinical interactions between conventional drugs and St John's wort extract have been described. An example of such is that interactions have been reported with cyclosporine, indinavir and nevirapine, which could be explained by the CYP3A4 inhibition since these drugs are metabolized by this cythocrome [112]. In short, *in vitro*, *in vivo* animal and human studies indicated that the St John's wort extract shows inhibitory/induction effects on the P450 system, whose different resulting effects may be species- and tissue-specific depending on the dose, route, duration of the administration, formulation and source of the herb. Thus it is necessary to know such information to prevent undesirable clinical consequences [12].

Ginkgo biloba is obtained from the *Ginkgo biloba Linne* tree and it is one of the most widely used herbal products [113]. Evidences suggest that G. biloba leaf extract can inhibit CYP1A2 and 2D6 activities [114]. Anecdotal evidence suggests that G. biloba leaf extract might also be an inhibitor or inducer of CYP3A4 activity. Little effect on CYP2D6 and a moderate inhibition of CYP2C9 have been observed [115]. Flavonol aglycones, isorhamnetin, kaempferol, and quercetin, present in the extract are responsible for the *in vitro* inhibition of the catalytic activity of human CYP1B1 [116]. However, the effects on P450 enzymes are not likely to lead to clinically significant drug interactions. So, it is important to note the potentially increased risk of bleeding when G. biloba is used with antiplatelets, anticoagulants or herbs with coumarin constituents.

Ginseng *(Panax ginseng)* is a popular herbal medicine used as neuroprotective, antioxidative, antifatigue, hypolipidemic, immunomodulatory and chemopreventive products, among others [117-119]. The composition of ginseng includes ginsenosides, sterols, vitamins, polycetylenes, ß-elemine, choline, and different flavonoids are also present. Different *in vitro* and *in vivo* studies have shown that ginseng can modulate various P450 enzymes [120,121]. Ginsenosides exhibit no inhibition or weak inhibition against human CYP3A4, 2D6, 2C9, 2A6, or 1A2 activities using human liver microsomes and cDNA expressed CYP3A4, however, their main intestinal metabolites show a wide range of inhibition of the P450-mediated metabolism. Thus, suggest that after oral administration, naturally occurring ginsenosides might influence hepatic P450 activity *in vivo via* their intestinal metabolites [122]. Although, effects are mainly attribut-

able to ginsenosides present in the product, but the effects of flavonoids must not be ruled out.

Grapefruit Juice

Studies performed with grapefruit juice show that drinking this juice modulates the P450 system, which has been associated with an increase of the oral bioavailability of a number of drugs [123-128]. *In vivo* studies demonstrated that its consumption increased the plasma half-lives of drugs such as caffeine, an effect attributed to the inhibition of CYP1A2 by naringin [129]. The effect of grapefruit juice on drug metabolism is most pronounced in drugs with a high first-pass metabolism (i.e. felodipine, amiodarone), in which it inhibits the first-pass metabolism of CYP3A4 substrates. Flavonoids present in the juice are the molecules responsible for the inhibition observed; for example, naringin has also been identified as a potent inhibitor of intestinal CYP3A4 [130, 131].

Cuban Natural Products Rich in Polyphenols

Cubans have used herbal products rich in polyphenols during decades. A standard aqueous stem bark extract of *Mangifera indica*, named Vimang, is used as phytomedicine. Vimang has antioxidant, anti-inflammatory and immune-modulatory properties [132-136]. Polyphenols are the main fraction present in the extract, including phenolic acids (gallic acid, 3-4-dihydroxy benzoic acid), phenolic esters (methyl gallate, propyl gallate, propyl benzoate), flavan-3-ols ((+)-catechin and (-)-epicatechin) and mangiferin [31, 32].

Vimang did not modify CYP2C, 2D1, 2E1, and 3A1 activity in rat hepatocytes, but it did change oxidations catalyzed by CYP1A2 and 2B1 (Fig. **5**). It also reduced CYP1A2 activity, which may be caused either by a reduction of CYP1A2 levels induced by Vimang or by direct interaction of some component(s) of the extract with the catalytic function of the enzyme. The marked concentration-dependent decrease in CYP1A2 activity observed in hepatocytes shortly incubated with Vimang revealed a direct interference at the activity level [137]. Subsequent studies, performed in human microsomes corroborated the inhibitory effects on CYP1A (IC_{50} = 60 μg/mL). Mangiferin (2-ß-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthen-9-one) isolated from the extract is involved in the main biological properties described to Vimang [2,134]. Other pharmacologic effects have been also described for this polyphenol: anticancer, antidiabetic, neuroprotective and antiviral activity [138,139]. Mangiferin showed inhibitory effects on CYP1A1/2 activity on rat hepatocytes and human microsomes, but in contrast to Vimang, mangiferin inhibits the activity of other P450 enzymes.

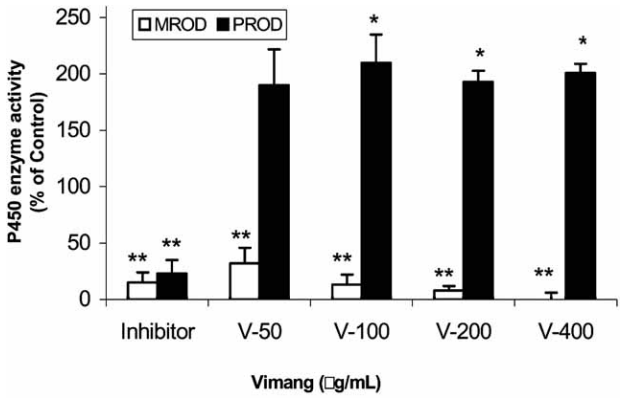

Fig. (5). Effects of *Mangifera indica* L. extract (Vimang) on 7-methoxyresorufin O-deethylation (MROD, CYP1A2) and 7-pentoxyresorufin O-depentylation (PROD, CYP2B1) activities in primary cultured rat hepatocytes. * $p < 0.05$, ** $p < 0.01$ (Wann Whitney test).

The genus *Erythroxylum* (Erythroxyceae) is widespread in tropical regions including Cuba, where there are 16 endemic species. They have been used in ethno-medical practice because their anti-inflammatory, anti-bacterial, antiviral and stimulant properties, for liver, renal and vesicular afflictions, among other [140,141]. In *Erythroxylum*, the prominent polyphenols are flavonols. Kaemferol, quercetin and ombuin are the main aglycones of *Erytroxylum* flavonols. Studies performed in Cuban on four endemic *Erytroxylum* species have found high concentration of flavonoids in leaf [35]. The effects of two leaf extracts: *Erythrolylum confusum* and *Erythrolylum minutifolium* on P450 system have been studied in cultured rat hepatocytes. Results suggested inhibitory action of both extracts on P450 enzymes (unpublished data).

Different plants rich in polyphenols are present in Cuban marine platform. *Thalassia* is one of these extracts prepared from aquatic plants with antioxidant, neuroprotective and anti-inflammatory effects [142]. Preliminary data from our laboratory show a light inhibition of several P450 activities by this natural extract, but more studies are needed to confirm these result.

The above-mentioned experimental data suggested the need to study possible drug-interactions of this kind of products used in traditional medicine. Conclusive evidence of the potential interactions of these natural products with other medicines used in human therapy can only be obtained from studies performed *in vivo* and clinical studies combined with *in vitro* results in order to exclude the inter-species differences between animals and human metabolism.

CONCLUSIONS

This review has concentrated on several examples of the effects that plant polyphenols, frequently consumed by humans as food or as part of medicinal herbs, have on the P450 system. It is clear that P450s are the core of many metabolism-based interactions observed in the clinical practice. Polyphenols can induce and/or inhibit these isoenzymes and, thereby, alter the response of the organism to other xenobiotics (as conventional drugs) or endogenous compounds. There are many factors which determine whether interactions will occur and examples of such are the plasma levels of the interacting compounds, their affinities for P450 enzymes, exposure time, and the presence of other xenobiotics. The clinical effects are harder to anticipate and some of the factors that determine them include the magnitude of the interaction, the therapeutic index and the pharmacokinetic profile of the compounds involved in the interaction (more in the case of a complex mixture such as herbal medicines). The

observed effects of natural products on drug metabolism may differ with plant species, the source, the environment, and the processing and storage conditions. One important problem is to distinguish between the biomarker(s) responsible(s) for the pharmacological activity and those components that affect the P450 system with clinical repercussions. All these aspects are evidence of the need to evaluate potential drug-interactions when a new natural product is introduced into human therapeutics.

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Received: 26 April, 2007 **Revised: 31 July, 2007** **Accepted: 01 August, 2007**