INHIBITORY EFFECT OF EURYCOMA LONGIFOLIA EXTRACT AND EURYCOMANONE ON HUMAN CYTOCHROME P450 ISOFORMS

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ABSTRACT

Objective: The aim of this study was to evaluate the inhibitory effect of E. longifolia extract on five human cytochrome P450 isoforms.

Methods: The inhibitory effect of E. longifolia standardized extract (TAF-273) on five human cytochrome P450 isoforms, namely CYP1A2, CYP3A4, CYP2C9, CYP2C19 and CYP2D6, was determined using a luminescence method. The effect of eurycomanone on cytochrome P450 isoforms CYP2C9 and CYP2C19 was examined using the same method. The final concentrations of E. longifolia extract tested were 0.01, 0.1, 1, 10, 100 and 1000 µg/mL, and eurycomanone was tested at concentrations ranging from 0.002 to 500 µM.

Results: The extract decreased CYP2C9 and 2C19 activities, with IC50 values of 710.20 µg/mL and 739.05 µg/mL, respectively. The IC50 of TAF-273's inhibitory action on CYP2D6, CYP3A4 and CYP1A2 could not be determined because at the highest concentration tested (1000 µg/mL) the activity of each enzyme was still greater than 50%. Eurycomanone (a marker compound in TAF-273) showed IC50 values of 47.33 µg/mL and 167.38 µg/mL in inhibiting the activities of CYP2C9 and 2C19, respectively.

Conclusion: This inhibitory study showed that a standardized extract of E. longifolia was not a potent inhibitor towards CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP1A2. On the other hand, eurycomanone (the marker compound) was a moderate inhibitor for CYP2C9 with an IC50 of 47.33 µM. The data indicated that E. longifolia extract did not potently inhibit any of the CYP isoforms, while eurycomanone was a moderate inhibitor of CYP2C9.

Keywords: Eurycoma longifolia jack, Inhibitory effect, Human cytochrome P450.

INTRODUCTION

Eurycoma longifolia Jack (E. longifolia) is one of the popular medicinal plants in Southeast Asia, including Indonesia and Malaysia. E. longifolia has many local names: in Brunei it is known as tungat ali, lang sia or pasak bumi; in Cambodia it is known as antoung sar or antong sar; in Thailand it is known as plaalai phuenk, hae phan chan or phiak; in Laos it is known as narn; in Vietnam it is known as Cay ba binh; in Indonesia it is known as beseng, bidara laut or pasak bumi; and in Malaysia it is known as bedara merah, bedara putih or tongkat ali [1, 2].

In Malaysia, Tongkat Ali (E. longifolia) has been claimed to improve the stamina of men during sexual activity, increase vitality and restore erection. As such, it is reputed to be an aphrodisiac. The herb is commonly taken as a decoction of roots in water. Nearly 200 Tongkat Ali products are available on the domestic Malaysian market, either in combination with other herbs or as a single preparation [3]. Many publications have revealed the aphrodisiac activity of E. longifolia, which has been used to enhance male virility during sexual activity [4, 5, 6, 7 & 8]. Many activity studies have been performed with regard to the many constituents that are contained in E. longifolia [9, 10, 11, 12, 13, 14, 15 & 16]. Hitherto, only a few studies have been published on herb-drug interactions. One study by Salman et al (2010) [17] about the effect of a E. longifolia water-based extract on the bioavailability of propranolol showed that the extract decreased Cmax (42%), reduced AUC (29%) and prolonged T1/2 (86%) of propranolol. The effect of a standardized extract of E. longifolia on aminopyrine metabolism in male and female rats has also been examined. The extract significantly increased aminopyrine metabolism in male and female rat hepatocytes and there was a different effect at the molecular level on aminopyrine metabolism in male rats compared to female rats [18, 19]. Purwanti et al. (2011) [20] studied the effect of E. longifolia extract on rosiglitazone metabolism. The results showed that the extract increased rosiglitazone metabolism significantly, especially in aged normal and diabetic male rats.

Herb-drug interactions follow modern pharmacological principles and are divided into pharmacodynamic and pharmacokinetic interactions [21, 22]. Herb-drug pharmacokinetic interactions appear through the influence of an herb on the absorption, distribution, metabolism or excretion of drugs. Concurrent use of herbal medicines may alter a drug's concentration by either inhibiting or inducing intestinal and hepatic drug-metabolizing enzymes, especially cytochrome P450s (CYP), and also drug transporters like P-glycoprotein (Pgp) [23, 24 & 25]. An herb may modulate the metabolic clearance of drug by inducing or inhibiting a specific CYP enzyme [26]. Many assessments have been performed to evaluate the effect of medicinal plants on drug interactions and their effects on cytochrome activities [27]. This study examined the effect of a standardized E. longifolia extract on human cytochrome P450 (CYP450).

MATERIALS AND METHODS

Materials

The standardized extract of E. longifolia (TAF-273) was provided by Prof. Chan Kit Lam from the School of Pharmaceutical Sciences, Universiti Sains Malaysia. The extract was made according to Chan et al. (2004) [10] and a voucher specimen of the plant was deposited, with Reference No. 785 -117, at the Penang Botanical Garden [1]. The E. longifolia extract was standardized with eurycomanone as a marker compound, using a MICROT_Of-QI LC-MS (Bruker ®, USA) and the eurycomanone content in the extract was found to be 15.89 % ± 0.95 SD. A luminescence assay was carried out as described in the P450-GLP™ Screening System by Promega®, USA and was performed in a 96-well microtiter plate (Thermo Scientific®, Finland). The screening system included the five subtypes of CYP450 (CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP1A2) and 5 different substrates used in these experiments were purchased from Promega®, USA: luciferin 6’ methyl ether (luciferin-ME) for CYP1A2, 6’ deoxyluciferin (luciferin-H) for CYP2C9, an ethylene glycol ester of 6’deoxyluciferin (luciferin-H-EGE) for CYP2C19, and an ethylene glycol ester of luciferin 6’ methyl ether
CYP2C19 activities are displayed in Fig. 1. The extract on CYP2C9 and *E. longifolia* The inhibitory effects of the activities. decreased CYP2C9 and CYP2C19 activities significantly, with an IC\textsubscript{50} of 710.20 µg/mL and 739.05 µg/mL, respectively; while TAF-273 could not be determined because, even at the highest concentration tested (1000 µg/mL), the activities of the enzymes were still more than 50 %.

**Enzyme assay**

A standard curve of CYP-P450 activity was prepared using a 2 mM D-luciferin stock solution. Then, a 4X serial dilution series of the D-luciferin standard (8 µM, 0.32 µM and 0.064 µM) was prepared to obtain standard solutions with final concentrations of 2 µM, 0.4 µM, 0.08 µM and 0.016 µM. The 4X CYP1A2, CYP3A4, CYP2C9, CYP2C19 or CYP2D6 reaction mixture, 4X control reaction mixture and 2X NADPH regeneration system were prepared before the assay was performed. Then, 12.5 µL of 4X D-luciferin standard was added into a 96-well white opaque plate in the appropriate wells and 12.5 µL of luciferin-free water was added to the 0 µM D-luciferin wells. Then 12.5 µL of the 4X control reaction mixture was added and the plate was shaken by tapping the plate. The reaction was pre-incubated for 10 minutes at 27 ºC and then 25 µL of the 2X NDPH regeneration system was added into each well. The plate was tapped and incubated at 27 ºC for 30 minutes. Then, 50 µL of the reconstituted luciferin detection reagent was added to each well, mixed briefly and incubated for 20 minutes at 27 ºC to stabilize the luminescent signal. The signal was recorded using a plate-reading luminometer (Hidex Plate CHAMELEON®, Hidex Oy, USA). Values were displayed as relative light units (RLU). To perform the test compound assay, 4X concentrations of the test compounds: *E. longifolia* solution or eurycomanone (12.5 µL) were added to the wells of a microtiter plate. In the untreated wells, the test compound was replaced with 12.5 µL of luciferin-free water. Then, 12.5 µL of the 4X control reaction mixture was added, the plate was shaken by tapping and the reaction was continued as mentioned above.

**Data analysis**

The activity was presented as a percentage of the activity of the control. Then, the IC\textsubscript{50} was calculated to determine the potency of the *E. longifolia* extract in inhibiting cytochrome P450 enzyme activities.

**RESULTS AND DISCUSSION**

The inhibitory effects of the *E. longifolia* extract on CYP2C9 and CYP2C19 activities are displayed in Fig. 1. The *E. longifolia* extract decreased CYP2C9 and CYP2C19 activities significantly, with an IC\textsubscript{50} of 710.20 µg/mL and 739.05 µg/mL, respectively; while eurycomanone (the marker compound) showed IC\textsubscript{50} values of 47.33 µM and 167.88 µM against CYP2C9 and CYP2C19 activities (Fig. 2).

The inhibitory effect of the *E. longifolia* extract on the activity of CYP2D6, CYP3A4 and CYP1A2 are presented in Fig. 3. The IC\textsubscript{50} of TAF-273 could not be determined because, even at the highest concentration tested (1000 µg/mL), the activities of the enzymes were still more than 50 %.

Cytochrome P-450 enzymes have an important role in the catabolism of endogenous substrates and the metabolism of most drugs and xenobiotics [28]. A drug may have a complex profile, i.e. it may be a substrate for an inducer and an inhibitor at the same time. For example, quinidine is known as a potent inhibitor of CYP2D6, but is mainly metabolized by CYP3A4 [29]. All CYPs may be inhibited or induced by exposure to a wide variety of xenobiotics, including various herbs.

Several studies related to herb interactions and CYP450 enzyme activities have been published [30]. Iwata et al. (2004) [31] reported that *Schisandra* extract was a potent inhibitor of erythromycin N-demethylation mediated by CYP3A4. Some isolated components from *Schisandra* fruit have also been identified as inhibitors of N-demethylation activity. Grapefruit juice has shown an inhibitory effect on CYP3A4 activity and some furanocoumarins in grapefruit juice are indicated as the causative components of the inhibition [32, 33 & 34]. Inhibitory studies of seven components of danshen extract (*Salvia miltiorrhiza*) on CYP450 activity showed that each component had a different effect. Some components were potent inhibitors of CYP1A2, one component was a competitive inhibitor of CYP2C9 and the others were weak inhibitors of CYP2D6 and CYP3A4 [35]. Another study by Obach (2000) [36] demonstrated that St. John’s wort extract inhibited CYP450 enzyme activities but the isolated biapigenin, hyperforin and hypericin presented different potencies. Biapigenin was a potent inhibitor of CYP2C9, CYP3A4 and CYP1A2. Hyperforin demonstrated strong inhibition of CYP2D6, CYP3A4 and CYP2C9 activities, while hypericin inhibited several CYP450 activities. These *in vitro* data suggest that St. John’s wort remedies should be evaluated for their *in vivo* potential for pharmacokinetic herb-drug interactions because these remedies contain constituents that may potently inhibit major drug metabolizing enzyme activities in humans. Hanapi et al. (2010) [37] evaluated the effects of five Malaysian medicinal plants on CYP2C9, CYP2D6 and CYP3A4. They concluded that three of the five examined plants (*M. speciosa, O. stamineus* and *A. paniculata*) showed herb-drug interactions, especially when administered concurrently.
with drugs that were metabolized by CYP2C9, CYP2D6 or CYP3A4. Inhibition or induction of CYP activities may influence drug metabolism and affect a drug's therapeutic effects. Thus, it may be beneficial or, on the other hand, cause adverse effects. The inhibitory effects of the major components of herbal remedies on CYP450 activities should be determined in order to predict interactions between drug and herbal remedies with regard to their metabolism. Their kinetics of inhibition and mechanism of action should also be identified [31]. Our results showed that E longifolia extract was not an inhibitor for CYP2D6, CYP3A4 and CYP1A2. The IC_{50} of the E longifolia extract's inhibitory activity could not be determined because the activity of the CYP isoforms were still greater than 50 % at the highest concentration tested. E longifolia extract is claimed to be an aphrodisiac by increasing testosterone levels [38, 39]. Increases in testosterone may increase the metabolic rate and oxidative phosphorylation. On the other hand, the inhibition studies of E. longifolia extract on CYP2C9 and CYP2C19 activities indicate that E longifolia extract is not a potent inhibitor of CYP2C9 and CYP2C19. Moreover, eurycomanone (the major compound in E longifolia extract) showed only a weak inhibitory potency for CYP2C9 and CYP2C19 activities because the IC_{50} values were greater than 1 μM [35]. From the pharmacotherapy perspective, this lack of a CYP modulatory effect is beneficial to patients, since E. longifolia is often consumed concurrently with antidiabetics, antihypertensives and other agents that may be CYP substrates or inhibitors [40].

CONCLUSION

An inhibitory study on the effect of a standardized extract of E. longifolia on human cytochrome P450 showed that the extract was not a potent inhibitor towards CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP1A2. On the other hand, eurycomanone (the marker compound) was a moderate inhibitor of CYP2C9 with an IC_{50} of 47.33 μM. This may beneficial for development of E. longifolia extract as a medicinal plant, since cytochrome P450 has an important role in drug metabolism and drug metabolism has a very close relationship with the therapeutic effects of drugs.

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REFERENCES


