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Selective inhibition of human cytochrome P450 1A2 by *Moringa oleifera*

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Abstract

The use of *Moringa oleifera* Lam as a healthcare product in Thailand becomes very popular during a past few years. Since medicinal plants constituents may interact with drug metabolizing enzymes, particular cytochromes P450 (CYP) raises the potential of herb-drug interactions. The aim of the present study was to determine the inhibitory effects of *M. oleifera* extractions on human CYP activities including CYP1A2, CYP2D6, CYP2E1 and CYP3A4 using selective substrate of these enzymes. The results revealed that both ethanolic and aqueous extracts of *M. oleifera* inhibited CYP1A2, CYP2D6, CYP2E1 and CYP3A4 activities in a dose-dependent manner. Moreover ethanolic extract of *M. oleifera* exhibited selective inhibition of CYP1A2 with IC_{50} values of 13.8 $\pm$ 9.8 μg/mL incubation. The results obtained from the present study suggested the possibility of potential herb and drug interactions of *M. oleifera*. Therefore, healthcare professionals and patients should be aware when using this medicinal plant with some prescribed drugs especially drugs that metabolized by CYP1A2.

Keywords: *Moringa oleifera*, Cytochromes P450, Herb-Drug interaction

Introduction

Moringa oleifera Lam (*M. oleifera*), commonly known in Thai as "ma-room", has long been used as foods, nutritional supplements, or medicines in several countries, including Thailand. *M. oleifera* is a tree belongs to Moringaceae family. It has been reported to have various pharmacological properties such as antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities (1). *M. oleifera* contains several compounds such as carotenoids, flavonoids, phenolics and ascorbic acid (2, 3). Several medicinal plants have been reported to inhibit the activities of cytochromes P450 (CYP) which are the most important enzymes involved in the metabolism of thousands of clinically used drugs. Major isoforms of human CYP isoforms that involved in drug metabolism are CYP3A4, CYP2D6, CYP2E1 and CYP1A2 (4). The potential interaction of medicinal plants with clinically used drugs is a major safety concern, especially for drugs with narrow therapeutic index (e.g. warfarin and theophylline) and may lead to treatment failure or life threatening adverse reactions (5).

Although *M. oleifera* has been used since ancient times in Thailand, data concerning the interaction between this plant and CYPs is still limited. The aim of the present study was to determine the inhibition effects of *M. oleifera* both ethanolic and aqueous extracts on human CYP1A2, CYP2D6, CYP2E1 and CYP3A4 activities.

Materials & Methods

Preparation of M. oleifera ethanolic and aqueous extract: Dried leaves of *M. oleifera* was grounded and extracted with 95% ethanol or water. Ethanol or water was evaporated under low pressure at 60°C and freeze dry, respectively.

Inhibitory effect of M. oleifera extracts on human CYP activities: Human hepatic phenacetin O-deethylase, dextromethorphan O-demethylase, chlorzoxazone 6-β hydroxylase and testosterone 6β-hydroxylase were used as a selective marker of CYP1A2, CYP2D6, CYP2E1 and CYP3A4, respectively (6, 7). An incubation mixture (500 μl) contained the ethanolic or aqueous extract of *M. oleifera*, a selective CYP substrate, human liver microsomes in 0.1 M phosphate buffer, pH 7.4. An equal volume of DMSO or water was also added in controls. The reaction was initiated by the addition of β-NADPH then incubated at 37 °C for a specific period. The amounts of metabolites were measured by HPLC.

Data analysis: IC_{50} values (concentrations of inhibitor causing 50% reduction in activity relative to the control) were calculated by linear regression analysis of the log inhibitor concentration versus percentage control activity plots.

Results

The ethanolic and aqueous leaves extracts of *M. oleifera* inhibited human CYP1A2, CYP2D6, CYP2E1 and CYP3A4 activities in dose-dependent manner with varied IC_{50} values range from 13.8 to 1,500 μg/mL incubation (Table 1).

Ethanolic extract of *M. oleifera* showed selective inhibition of CYP1A2 with IC_{50} value of 13.8 μg/mL incubation. Comparing to the known CYP1A2 inhibitors, the inhibitory potency of *M. oleifera* ethanolic extract on CYP1A2 was less than furafylline but more potent than cimetidine.

CYP 3A4 was also inhibited by the extracts of *M. oleifera* which the ethanolic extract exhibited comparatively stronger inhibitory activity towards CYP3A4 than clarithromycin, with IC_{50} value of 101 μg/mL incubation.

Table 1: IC_{50} values of *M. oleifera* ethanolic and aqueous extract on human CYP1A2, CYP2D6, CYP2E1 and CYP3A4 activities.

	IC_{50} values (μg/mL incubation)			
	CYP1A2	CYP2D6	CYP2E1	CYP3A4
Known CYP1A2 inhibitor				
Furafylline	0.08 ± 0.05			
Cimetidine	71.0 ± 52.8			
Known CYP2D6 inhibitor				
Quinidine		0.97 ± 0.06		
Fluoxetine		0.04 ± 0.01		
Paroxetine		0.02 ± 0.01		
Known CYP2E1 inhibitor				
Diethyldithiocarbamate			0.18 ± 0.05	
Disulfiram			0.83 ± 0.21	
Known CYP3A4 inhibitor				
Ketoconazole				0.10 ± 0.08
Erythromycin				83.3 ± 61.1
Clarithromycin				730 ± 233
Moringa oleifera				
Ethanolic extract	13.8 ± 9.8	219 ± 114	> 400[a]	101 ± 40
Aqueous extract	630 ± 324	> 1000[b]	725 ± 243	1500 ± 258

Data presents as mean ± S.D. from four microsomal preparations.

[a] The data was limited by the solubility of *M. oleifera* ethanolic extract.

[b] The peak of dextromethorphan O-demethylation was interfered by the high concentration of *M. oleifera* aqueous extract.

Discussion and conclusion

Both ethanolic and aqueous extracts of *M. oleifera* inhibited human CYP1A2, CYP2D6, CYP2E1 and CYP3A4 activities *in vitro* in a dose-dependent manner. *M. oleifera* particularly the ethanolic extract was a strong inhibitor of human CYP1A2. CYP1A2 is the main hepatic CYP1A which involved in the metabolisms of a number of clinically used drugs such as acetaminophen, alosetron, theophylline, tarcrine, tizanidine etc.

This results obtained from the present study suggest the possibility of potential herb-drug interaction if *M. oleifera* was administrated concomitantly with medicines that are metabolizes by CYP1A2. The effects of this medicinal plant *in vivo* need further investigation.

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