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Research Article

TAMARIND EXTRACT INHIBITS CYTOCHROME P450 (CYP3A4 ISOZYME) - AN *IN VITRO* STUDY

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ABSTRACT

Objective: The aim of the present study was to analyze the effect of the aqueous fruit pulp extract of *Tamarindus indica* L. (tamarind extract) on cytochrome P 450 isoform CYP3A4.

Methods: Tamarind extract at different concentrations from 5 to 100 µg/ml was examined for its inhibitory property toward cytochrome P 450 isoform CYP3A4. The various concentrations of tamarind extract, potassium phosphate buffer, CYP450 reagent, and substrate 7-Benzyloxy-4-trifluoromethylcoumarin were added to a 96-well plate. The mixtures were preincubated for 20 min at room temperature. The reaction was started by a mixture of free constituted substrate and $NADP^+$ and incubated at room temperature for 30–60 min. The reaction was stopped by Tris-HCl buffer, pH 10.5. The fluorescent intensities of the products were measured by PerkinElmer Enspire fluorescence reader using an excitation and emission wavelength of 405 nm and 460 nm, respectively. Inhibitory concentration (IC_{50}) was calculated by plotting concentrations of tamarind extract against the corresponding percentage inhibition.

Results: All the tested concentrations of extract except 5 µg/ml showed good inhibition against CYP3A4 in a dose-dependent manner. The IC_{50} value of tamarind for CYP3A4 inhibitory activity was found to be 27.89 µg/ml.

Conclusion: *T. indica* aqueous fruit pulp extract exhibited an inhibitory effect on CYP34A, thereby indicating the possibilities of herb-drug interaction if these extracts are coadministered with the prescribed drugs that are metabolized by CYP3A4.

Keywords: Tamarind extract, Cytochrome P450 enzymes, Herb-drug interaction, Inhibition.

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INTRODUCTION

Cytochrome P450 genes are involved in the formation and breakdown of various molecules and chemicals within cells [1]. They play an important role in the synthesis of many molecules including steroid hormones, certain fats, and acids used to digest fats in the human body. It represents a family of isozymes responsible for the biotransformation of many drugs through oxidation [2]. Majority of the isozymes are located in the liver. However, extrahepatic metabolism occurs in the kidney, skin, gastrointestinal tract, and lungs.

Medicinal plants are a rich source of bioactive natural products in most precise and most selective way. Since the mid-19th century, many natural products have been purified from plants, and most of them are used as active elements of the modern medication. The search of excellent, real, inexpensive, and simply accessible natural enzyme inhibitor is one of the prime aspects of new drug findings and strategy investigation works which are done by the study organizations throughout the world [3]. Plants and herbs have been widely consumed in many populations of the world mainly as food and for various medicinal benefits. Among the various types of bioactive compounds found in plants and herbs, phenolic compounds that are secondary metabolites have been widely reported as potent antioxidants.

Tamarindus indica which is known as the tamarind tree is a tropical tree growing abundantly in tropical rain forests. It is known to possess high antioxidant activity due to its phenolic content. The various parts of the plant are used for its antimicrobial, hepatoprotective, anticancer, analgesic, anti-inflammatory, and antiasthmatic property [4,5]. In African sub-Saharan populations, T. indica is reported to be the most useful tree which serves as ethnomedicine for humans and veterinary field and also has cultural uses [6].

When drugs are administered orally, some of them are significantly inactivated due to the extensive first-pass metabolism in the gastrointestinal tract by the CYP3A4 isozyme. The drug breakdown which takes place through the cytochrome P450 system has emerged as an important determinant in the occurrence of several drug-drug interactions. A greater degree of drug interaction predictability has been achieved through the identification of the P450 isozymes and a few other drugs that share them. CYP3A4 isozyme has resulted in several clinically significant drug-drug interactions. In this study, the aqueous fruit pulp extract of tamarind was tested for its effect on cytochromeP450 (CYP3A4) isozyme.

METHODS

Plant material

The aqueous fruit pulp extract of tamarind was obtained from Synthite Industries Pvt. Ltd., Kerala, as a gift sample.

Chemicals

CYP450 reagent, 7-Benzyloxy-4trifluoromethylcoumarin (BFC), Tris-HCl buffer, and potassium phosphate buffer were used. All the chemicals used were of analytical grade.

Inhibitory effect of cytochrome P 450 enzyme activity (CYP3A4)

Tamarind extract at different concentrations from 5 to 100 µg/ml was examined for its inhibitory property toward cytochrome P 450 isoform CYP3A4. The various concentrations of tamarind, potassium phosphate buffer, CYP450 reagent, and substrate BFC were added to a 96-well plate. The mixtures were preincubated for 20 min at room temperature. The reaction was started by a mixture of free constituted substrate and $NADP^+$ and incubated at room temperature for 30–60 min. The reaction was stopped by Tris-HCl buffer, pH 10.5. The fluorescent intensities

of the products were measured by PerkinElmer Enspire fluorescence reader using an excitation and emission wavelength of 405 nm and 460 nm, respectively [7]. Inhibitory concentration (IC_{50}) was calculated by plotting concentrations of tamarind extract at different concentrations from 5 to 100 µg/ml which was examined against the corresponding percentage inhibition. Values are expressed as mean ± standard error of the mean (n=3).

RESULTS

All the tested concentrations of extract except 5 µg/ml showed potent inhibition against CYP3A4 in a dose-dependent manner. The IC_{50} value of tamarind for CYP3A4 inhibitory activity was found to be 27.89 µg/ml. The extract showed a dose dependant inhibitory effect on different concentrations. At 10 µg/ml, the percentage inhibition was found to be 5.70±4.08; at 20 µg/ml, it was found to be 18.35±2.75; at 40 µg/ml, it was found to be 27.12±0.20; at 60 µg/ml, it was found to be 39.77±0.57; at 80 µg/ml, it was found to be 50.00±0.28; and at 100 µg/ml, it was found to be 62.49±0.65 (Fig. 1).

DISCUSSION

In this study, the extract showed a dose-dependent inhibitory effect on CYP3A4 showing the possibility of drug interaction when administered with drugs which are metabolized by this particular enzyme and the IC_{50} value of tamarind extract was found to be 27.89 µg/ml.

The cytochrome p450 enzymes play a vital role in the Phase 1 metabolism, and they are involved in the detoxification, oxidation, and elimination of toxins and drugs. Many plants and fruits are proven to produce various enzyme inhibitory effect. Grapefruit and star fruit extracts are few examples of fruit extracts causing potent inhibition of cytochrome P450 enzymes [8,9].

The extracts of *Hyptis suaveolens* (HS) and *Boerhavia diffusa* (BD) which are medicinal plants used widely to treat eczema, boils, and malaria were shown to produce activity on drug metabolizing enzymes, CYP1A2, CYP2D6, and CYP3A4. BD was found to be the most potent on CYP3A4 (7.36±0.94 µg/mL) compared to both CYP2D6 (17.79±1.02 µg/mL) and CYP1A2 (9.48±0.78 µg/mL) in comparison with HS CYP1A2 (3.68±0.10 µg/mL) being the least inhibited by HS compared to CYP2D6 (1.39±0.01 µg/mL) and CYP3A4 (2.36±0.57 µg/mL) [10].

In a study by Higdon and Frei, catechin and epicatechin detected in tea prevented oxidative stress in rats' liver by directly altering the subcellular ROS production, glutathione metabolism, and cytochrome P450 2E1 activity [11]. *Eugenia jambolana* Lam fruit is very popular for its antidiabetic property, and in a study, it showed its effect on various cytochrome P450 enzymes. It showed the differential effect on cytochrome P450 activities with an order of inhibitory potential as CYP2C9>CYP3A4>CYP2D6 having IC_{50} of 76.69, 359.02, and 493.05 µg/ml [12]. In earlier studies, the data were consistent compared to the IC_{50} of the selective inhibitors of our study to the particular CYPs reported earlier [6,13-17]. The concentration-inhibition curves of the medicinal plants *Phyllanthus amarus* and *Phyllanthus emblica* aqueous extracts on CYP3A4 activities were observed that *P. amarus* aqueous extract possessed inhibitory effects on CYP3A4 with IC_{50} of 74.30±1.21, 34.80±0.34, 180.40±2.53, 49.41±0.52, and 2.07±0.03 µg/mL, respectively. *P. emblica* aqueous extract demonstrated inhibitory effects on CYP3A4 with IC_{50} of 310.27±5.07, 194.72±2.94, 589.52±14.32, 310.27±15.06, and 325.54±7.44 µg/ml. In our study, we also found that the IC_{50} value of tamarind for CYP3A4 inhibitory activity was found to be 27.89 µg/ml and showed increased inhibitory activity as the concentration of the aqueous tamarind fruit pulp extract was added in increments.

Apart from intake of a product and looking into its safety and effectiveness, attention should be paid to the potential that these product ingredients may interact with medications [17,18] as many plant products are useful in treating various disease conditions

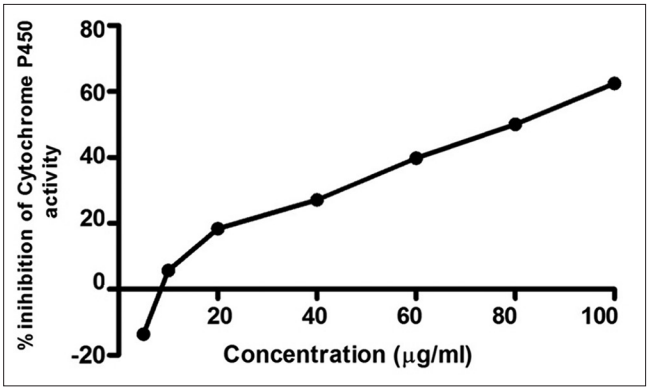

Fig. 1: The effect of tamarind extract on cytochrome P450 (CYP3A4)

such as diabetes mellitus, anxiety, cancer, and many other health problems [19-22]. Hence, proper care should be taken to administer such products after confirming its effect on various metabolizing enzymes. In developing countries, a large population depends on herbal medicine for prevention, cure, and mitigation of disease. The simultaneous use of herbs may mimic, magnify, or oppose the effect of drugs. It is necessary to conduct such studies to avoid unwanted drug interactions.

Herbals are capable of interacting with prescribed medications when they are taken together at the same time and can lead the patients to high risk. The interactions include drug metabolizing enzymes and drug transporters. Interactions between herbal remedies and drugs lead to the loss of therapeutic efficacy and toxic effects. Pharmacists and other health-care providers must be taking an active role in learning about herbals and other dietary supplements to avoid herb-drug interactions [23]. Precautions must be taken as various extracts and juices which are consumed daily life to avoid inhibitory potential toward these enzymes [24]. In addition, any suspected herbal drug interactions should immediately be reported to the Food and Drug Administration's Adverse Event Reporting Program.

CONCLUSION

The aqueous fruit pulp extract of *T. indica* exhibited an inhibitory effect on CYP34A, thereby indicating the possibilities of herb-drug interaction if these extracts are coadministered with the prescribed drugs that are metabolized by CYP3A4.

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AUTHOR'S CONTRIBUTION

The authors have equally contributed to the study. The work as well as the manuscript was done by R. Jagadish Rajkumaar under the guidance of Dr. Anitha Roy and Dr. Lakshmi T.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest in publishing in this paper.

REFERENCES

1. Anzenbacher P, Anzenbacherova E. Review cytochrome P450 and metabolism of xenobiotics. Cell Mol Life Sci 2001;58:737-47.
2. Marks BD, Larson BR. Miniaturization and automation of cytochrome

P450 inhibition Assay. Acta Pharm Sin B 2012;2:137-45.
3. Uddin G, Rauf A, Siddiqui BS, Shah S. Preliminary comparative phytochemical screen-ing of Diospyros lotus Stewart. Mid J Sci Res 2011;10:78-81.
4. Razali N, Aziz AA, Lim CY, Junit SM. The investigation into the effects of an antioxidant-rich extract of Tamarindus indica leaf on antioxidant enzyme activities, oxidative stress and gene expression profiles in HepG2 cells. Peer J 2015;3:e1292.
5. Ranjan R, Swarup D, Patra RC, Chandra V. Tamarindus indica L and Moringa oleiferaoleifera M. Extract administration ameliorates fluoride toxicity in rabbits. Indian J Exp Biol 2009;47:900-5.
6. Ebifa-Othieno E, Mugisha A, Nyeko P, Kabasa JD. Knowledge, attitudes, and practices in tamarind (Tamarindus indica L) use and conservation in Eastern Uganda. J Ethnobiol Ethnomed 2017;13:5.
7. Anannarukan N, Niwattisaiwong N, Warisnoicharoen W, Winitthana T, Pramyothin P, Chaichantipyuth C, et al. Inhibition of human cytochrome P450 in vitro by Phyllanthus amarus and Phyllanthus emblica aqueous extracts. Thai J Pharm Sci 2012;36:135-43.
8. Zhang JW, Liu Y, Cheng J, Li W, Ma H, Liu HT, et al. Inhibition of human liver cytochrome P450 by star fruit juice. J Pharm Pharm Sci 2007;10:496-503.
9. Ashour ML, Youssef FS, Gad HA, Wink M. Inhibition of cytochrome P450 (CYP3A4) activity by extracts from 57 plants used in traditional Chinese medicine (TCM). Pharm Mag 2017;13:300-8.
10. Thomford NE, Dzobo K, Adu F, Chirikure S, Wonkam A, Dandara C.Bush mint (Hyptis suaveolens) and spreading hogweed (Boerhavia diffusa) medicinal plant extracts differentially affect activities of CYP1A2, CYP2D6, and CYP3A4 enzymes. J Ethnopharmacol 2018;211:58-69.
11. Higdon JV, Frei B. Tea catechins and polyphenols: Health effects, metabolism and antioxidant functions. Crit Rev Food Sci Nutr 2010;43:89-143.
12. Chinni S, Dubala A, Kosaraju J, Khatwal RB, Satish Kumar MN, Kannan E. Effect of crude extract of Eugenia jambolana Lam. on human cytochrome P450 enzymes. Phytother Res 2014;28:1731-4.
13. Sripanidkulchai B, Tattawasart U, Laupatarakasem P, Vinitketkumneun U, Sripanidkulchai K, Furihata C, et al. Antimutagenic and anticarcinogenic effects of Phyllanthus amarus. Phytomedicine 2002;9:26-32.
14. Muto S, Fujita K, Yamazaki Y, Kamataki T. Inhibition by green tea catechins of metabolic activation of procarcinogens by human cytochrome P450. Mutat Res 2001;479:197-206.
15. Winitthana T, Niwattisaiwong N, Patarapanich C, Tantisira MH, Lawanprasert S. In vitro inhibitory effects of asiaticoside and madecassoside on human cytochrome P450. Toxicol In Vitro 2011;25:890-6.
16. Marks BD, Smith RW, Braun HA, Goossens TA, Christenson M, Ozers MS, et al. A high throughput screening assay to screen for CYP2E1 metabolism and inhibition using a fluorogenic Vivid P450 substrate Assay. Drug Dev Technol 2002;1:73-81.
17. Marks BD, Larson BR. Miniaturization and automation of cytochrome P450 inhibition assay. Available from: http://www.tools.invitrogen.com/downloads/Miniaturization Automation Cytochrome P450.pdf. [Last cited on 2011].
18. Kumar KB, Kuttan R. Inhibition of drug metabolizing enzymes (cytochrome P450) in vitro as well as in vivo by Phyllanthus amarus Schum and Thonn. Biol Pharm Bull 2006;29:1310-3.
19. Ashwini S, Ezhilarasan D, Anitha R, Cytotoxic effect of Caralluma fimbriata against human colon cancer cells. Pharm J 2017;9:98-101.
20. Ashwini S, Anitha R. Anti-hyperglycaemic activity of Caralluma fimbriata-An in vitro approach. Pharm Magaz 2017;13:S499-544.
21. Anitha R, Geetha RV, Lakshmi T, Nallanayagam M. Edible fruits - nature's gift for diabetic patients - a comprehensive review. Int J Pharm Sci Rev Res 2011;9:170-80.
22. Sarah S, Dhanraj G, Anitha R. Anxiety and aromatherapy- a review. J Pharm Biosci 2016;7:267-73.
23. Ismail MY. Herb-drug interactions and patient counselling. Int J Pharm Pharm Sci 2009;1:151-61.
24. Patil L, Kulkarni K, Khanvilkar V, Kadam V. In vitro evaluation of herb-drug interactions: A review. Int J Pharm Pharm Sci 2014;6:9-12.