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Research Article

EVALUATION OF THE CYTOCHROME P450 INHIBITORY EFFECT OF THYME OLEORESIN FROM *THYMUS VULGARIS L.* - AN *IN VITRO* STUDY

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ABSTRACT

Objective: The objective of this study was to evaluate the effect of thyme oleoresin on cytochrome P450 (CYP3A4) enzyme.

Materials and Methods: The different concentrations of thyme oleoresin (5–100 µg/ml) were examined for its inhibitory property toward cytochrome P450 isoform (CYP3A4). Thyme oleoresin, 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 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. Values were expressed as mean ± standard error mean (n=3). IC_{50} was calculated by plotting concentrations of thyme against the corresponding percent inhibition.

Results: All the tested concentrations of thyme showed inhibitory effect against CYP3A4 in a dose-dependent manner. At 5 µg/ml, it showed a percentage inhibition of 1.82±0.61, whereas 100 µg/ml showed 66.05±0.16. The IC_{50} value of thyme for CYP3A4 inhibitory activity was found to be 39.14 µg/ml.

Conclusion: This study prove the inhibitory effect of thyme oleoresin on cytochrome P450. The inhibitory effect of thyme oleoresin indicates the possibilities of herb-drug interaction if this extract is co-administered with prescribed drugs that are metabolized by CYP3A4.

Keywords: Thyme oleoresin, Cytochrome P450, Inhibitory assay, *Thymus vulgaris.*

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INTRODUCTION

Plants are extensively explored to evaluate their antibacterial activity, antioxidant activity as well as their effect in various metabolic diseases and cancer. [1-4]. Oleoresins are composed of resins and essential oils obtained from herbs [5,6] and are rich in antioxidants [7-9]. They are extensively used in pharmaceutical and food industries [10-13]. In this study, thyme oleoresin from *Thymus vulgaris L.* was used for evaluating its effect on cytochrome P450 (CYP3A4).

Cytochrome P450 is an important determinant in drug metabolism as well as in the occurrence of several drug interactions. These drug interactions can result in therapeutic failure, adverse drug reactions, and drug toxicities. Clinically, significant interactions can be prevented by identifying the drug involved as an enzyme substrate, inducer, or inhibitor and avoiding the coadministration of such drugs to get optimum response for the drugs [14].

Cytochrome P450 enzymes are primarily found in liver cells but are also located in cells throughout the body. Cytochrome P450 enzymes are located in endoplasmic reticulum and mitochondria. The enzymes found in mitochondria are generally involved in the synthesis and metabolism of internal substances, while enzymes in the endoplasmic reticulum usually metabolize external substances, primarily medications, and environmental pollutants.

CYP3A4 isozymes are responsible for the extensive first-pass metabolism and inactivation of some drugs which are administered orally [15,16]. It is reported that, among the six isozymes of P450 such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, several clinically significant drug-drug interactions have resulted from CYP3A4 isozyme [17,18].

MATERIALS AND METHODS

Plant material

The thyme oleoresin from marigold flowers was obtained from Synthite Industries Private Limited, Kerala, as a gift sample.

Chemicals

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

Inhibitory effect of cytochrome P450 enzyme activity (CYP3A4)

About 5–100 µg/ml of the thyme oleoresin was used to evaluate the cytochrome P450 isoform CYP3A4 inhibitory effect. The various concentrations of thyme oleoresin, 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. IC_{50} was calculated by plotting concentrations of thyme from 5 to 100 µg/ml against the corresponding percentage inhibition. [19].

RESULTS

In this study, all the tested concentrations of thyme showed inhibitory effect against CYP3A4 in a dose-dependent manner. At 5 µg/ml, it showed a percentage inhibition of 1.82±0.61, whereas 100 µg/ml showed 66.05±0.16. The IC50 value for CYP3A4 inhibitory activity was found to be 39.14 µg/ml (Table 1).

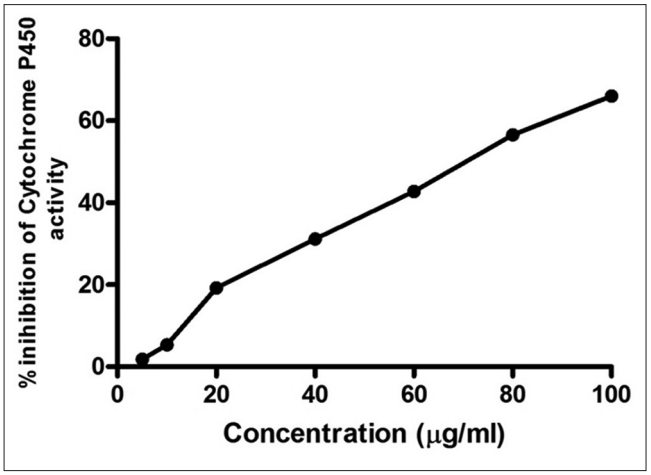

Fig. 1: Graph showing the effect of thyme oleoresin on cytochrome P450 (CYP3A4) inhibitory potential

Table 1: Effect of thyme oleoresin on cytochrome P450 (CYP3A4) inhibitory potential

Concentration (μg/ml)	Thyme oleoresin
5	1.82±0.61
10	5.38±0.44
20	19.20±0.36
40	31.16±0.20
60	42.76±0.32
80	56.55±0.77
100	66.05±0.16

Values are expressed as mean±SEM (n=3)

DISCUSSION

The present study shows dose-dependent inhibitory effect of thyme oleoresin on CYP3A4. (Fig 1) The IC_{50} value of thyme for CYP3A4 inhibitory activity was found to be 39.14 μg/ml. Cytochrome P450 is a superfamily of hemoproteins which can be divided into families, subfamilies, and/or single enzymes. The cytochrome P450 enzymes act as a major catalyst for drug oxidation. It is the most important enzyme involved in Phase I reaction. Each enzyme of the CYP family is termed as an isoform or isoenzyme, as it is derived from different genes. CYP3A4 is an isoenzyme which is an important subset of the cytochrome P450 family which accounts for nearly 60% of the total CYP450 in the liver and approximately 70% in the intestine. The biotransformation of many drugs is catalyzed by CYP3A and is significantly expressed extrahepatically [19].

Not only endogenous and exogenous factors but also the dietary and environmental factors can influence and affect cytochrome P450 enzyme activities [20]. Drugs can induce or inhibit cytochrome P450 and can cause clinically significant drug-drug interactions, unanticipated adverse reactions, or therapeutic failures. Interactions with many drugs such as warfarin, antidepressants, antiepileptic drugs, and statins often involve the cytochrome P450 enzymes. The possibility of adverse drug reactions and interactions can be minimized by having knowledge of the most important drugs metabolized by cytochrome P450 enzymes, as well as the most potent inhibiting and inducing drugs [21]. The high level of CYP3A4 expression in the small intestine may be the reason for CYP3A4-related interaction of food components, and moreover, more than 50% of clinical pharmaceuticals are metabolized by CYP3A4 [22].

Many plants such as *Terminalia chebula* and *Echinacea purpurea* are known for its cytochrome p450 enzyme inhibitory effect [23,24]. Traditional Chinese plants such as *Andrographis paniculata*, *Arctium lappa*, *Acacia catechu*, *Bupleurum marginatum*, *Dysosma versipellis,* and *Spatholobus suberectus* are also reported to have CYP3A inhibitory effect because of the presence of polyphenolics and suggests that these plants can interfere with the metabolism of concomitantly administered herbs or drugs which are metabolized by CYP3A4 [25].

Studies have even correlated the phytoconstituents responsible for cytochrome inhibitory effect. It is reported that *Gynura procumbens* extract with the highest content of flavonoids showed the highest inhibition of CYP3A4 and CYP1A2 enzyme activities. The ethanol extract of *G. procumbens* revealed the most potent inhibitory effect towards CYP3A4 and CYP1A2 enzyme while the methanol extract exhibited moderate inhibitory effect [26].

In the present scenario, a large population is depending on herbal medicine for a variety of health conditions such as common cold, inflammatory conditions, central nervous system diseases, heart disease, and diabetes, but their safety and efficacy data are not adequate [27,28]. Hence, it is always good to explore the possibility of such drug interaction for better clinical efficacy.

CONCLUSION

The findings of this study revealed that thyme oleoresin has the ability to inhibit cytochrome P450 enzyme activity, specifically CYP3A4. Hence, administration of thyme oleoresin together with herbal or modern drugs which follow the same metabolic pathway may result in herb-drug interactions.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in publishing this article.

AUTHOR'S CONTRIBUTION

All the authors have equally contributed towards the compilation of this research article.

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