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Original Article

Effect of Lutein on Cytochrome P450 (Isoform CYP3A4) - An *in vitro* Study

Murthy Meenapriya[1], Roy Anitha[2*], Thangavelu Lakshmi[2]

ABSTRACT

Background: Lutein is a carotenoids vitamin rich in many fruits and vegetables and also available in multivitamin products. It is consumed for its effect on eye disease, cancer, diabetes and other health conditions. Recently, herbal preparations are increasingly used in healthcare systems and concomitant administration of synthetic medications may cause pharmacokinetic or pharmacodynamic interactions leading to very serious medical problems. Understanding the ability of herbal extracts and preparations to modulate the metabolizing enzymes can help the health system for proper treatment of patients and thereby can avoid many adverse effects associated with it. The aim of the study was to find the effect of lutein isolated from *Tagetes erecta* L on cytochrome P450 isoform CYP3A4. **Materials and Methods:** The different concentrations of lutein (5 – 100µg/ml), potassium phosphate buffer, CYP450 reagent and substrate 7-Benzyloxy-4-trifluoromethylcoumarin (BFC) were added to a 96-well plate. The fluorescent intensities of the products were measured by Perkin Elmer Enspire fluorescence reader using an excitation and emission wavelength of 405 nm and 460 nm, respectively to examine the effect of lutein on Cytochrome P 450 isoform CYP3A4 and the IC_{50} was calculated by plotting concentrations of lutein against the corresponding percent inhibition. **Results:** All the tested concentrations of lutein showed potent inhibition against CYP3A4 in a dose – dependent manner. The IC_{50} value was found to be 35.27µg/ml. C**onclusion:** The inhibitory effect of lutein indicates the possibilities of herb-drug interaction if it is co – administered with prescribed drugs that are normally metabolised by CYP3A4 enzyme.

Key words: Lutein, Cytochrome P450, Inhibitory assay, Marigold, CYP3A4.

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INTRODUCTION

Lutein from *Tagetes erecta L.* is a purified extract obtained from marigold, which is extracted from the petals of marigold flowers. Lutein is chemically 3R,3'R,6'R-βε-carotene-3,3'-diol, which is a member of a group of pigments known as xanthophyll. It is used as a food colouring agent and nutrient supplement in a wide range of baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes in levels ranging from 2 to 330 mg/kg.[1]

Marigold flower (*Tagetes erecta* L. Family *Compositae*) represents a rich source of lutein. It is grown for business purposes in Mexico, Peru, Ecuador, Spain, India or China. Dried Marigold flowers contain 0.1–0.2% dry matter of carotenoids, out of which 80% are lutein diesters. By the extraction of dried and ground flowers, a non-polar oleoresin extract is acquired.[2] The recent evidence suggests that lutein is one of the abundant carotenoids in the diet and it possesses strong antioxidant capacity and may be useful in reduction of incidence of cancer.[3]

The xanthophylls, a major group of carotenoids, primarily include astaxanthin, b-cryptoxanthin, canthaxanthin, lutein, and zeaxanthin.[4] Unpredicted drug interactions have led to severe adverse effects or treatment failures. Many of these interactions involve the inhibition or induction of drug-metabolising cytochrome P450 (CYP) enzymes. Similarly, dietary supplements or nutrients may be inhibitors or inducers of CYP enzymes and influence the pharmacokinetics of any co-administered drugs. There are few reports about interactions between drug-metabolising enzymes and astaxanthin, b-cryptoxanthin, canthaxanthin, lutein, and zeaxanthin.[5] the aim of the present study was to find the effect of lutein isolated from *Tagetes erecta* L on cytochrome P450 isoform CYP3A4.

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MATERIALS AND METHODS

Chemicals and Extract

Potassium phosphate buffer, CYP450 reagent, 7-Benzyloxy-4-trifluoro-methylcoumarin (BFC), Tris-HCl buffer. All the chemicals used were of analytical grade. Lutein extract was obtained from Synthite Industries Limited as gratis.

5-100 microgram concentrations of lutein, potassium phosphate buffer, CYP450reagent and substrate 7-Benzyloxy-4-trifluoromethylcoumarin (BFC) were added to a 96-well plate. The mixtures were pre - incubated for 20 min at room temperature. The reaction was started by a mixture of reconstituted 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 Perkin Elmer Enspire fluorescence reader using an excitation and emission wavelength of 405 nm and 460 nm, respectively.[6] IC_{50} was calculated by plotting concentrations of lutein against the corresponding percent inhibition. All tests were done in triplicate and values were expressed as mean ± SEM.

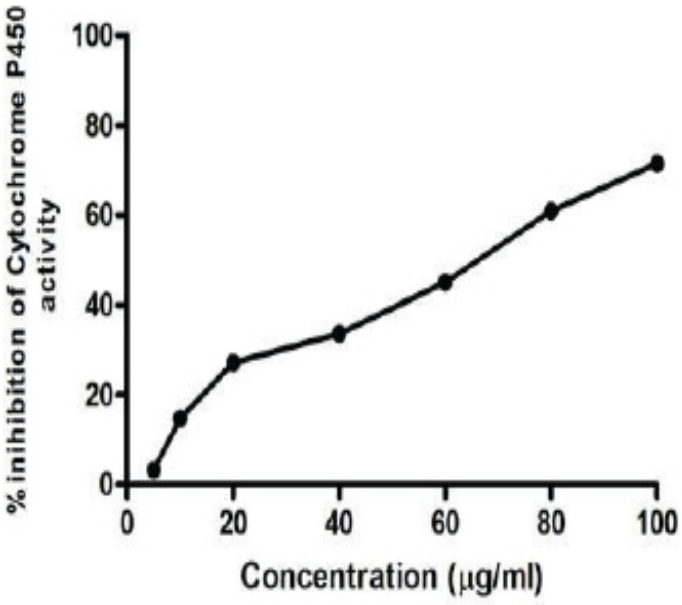

Figure 1: Inhibitory effect of Lutein on Cytochrome P450 Activity.

Table 1: Inhibitory effect of Lutein on Cytochrome P450 Activity.

Lutein concentration (µg/ml)	CYP450 inhibitory effect (%)
5	3.27 ± 0.69
10	14.83 ± 4.16
20	27.24 ± 0.16
40	33.71 ± 0.16
60	45.07 ± 1.17
80	60.95 ± 0.49
100	71.54 ± 0.49

RESULTS

All the tested concentrations (5 – 100µg/ml) of lutein showed good inhibition against CYP3A4 (Table 1 ,Figure1). The IC_{50} value of lutein for CYP3A4 inhibitory activity was found to be 35.27µg/ml.

DISCUSSION

In the present study, lutein extracted from marigold has demonstrated good inhibitory effect on CYP3A4. Results indicate the possibilities of herb-drug interaction if lutein is co-administered with the prescribed drugs that are metabolized by CYP3A4. Lutein is known for its antioxidant activity.[7]

The cytochrome P450 enzymes are the main players in Phase I metabolism and also involved in the oxidation and elimination of a wide array of xenobiotics such as drugs and toxins.[8] A number of therapeutic agents such as quinidine, vinblastine, ergotamine, berberine, and colchicines synthetic agents such as nifedipine and diazepamare the main substrates of CYP3A4.[9, 10] Many plant extracts are capable of inhibiting CYP3A4 activity.[11, 12] Grapefruit and kava-kava are best examples for herbal drugs that can cause clinically important modulation in CYP3A4 activity.[13, 14] Many other plants are also evaluated for their effect on CYP450 enzymes. The effect of *P. amarus* and *P. emblica* aqueous extracts on all CYP isoforms were weaker. In a previous study, extract of *Centenella asiatica* showed concentration-dependent inhibitory effect on CYP3A4 activities.[15] Studies show that plant products such as asiaticoside and madecassoside have inhibitory effect on CYP3A4. Asiaticoside inhibited CYP3A4 with an IC_{50} value of 3 43.35 ± 29.35µ M.Madecassoside, inhibited CYP3A4 with IC_{50} = 453.32 ± 39.33µ M.Asiaticoside exhibited non-competitive inhibition of CYP3A4 Madecassoside also showed non-competitive inhibition of CYP3A4.[16]

The inhibition of cytochrome P450 metabolism by blended herbal products and vitamins has revealed that the extracts have a low to moderate capacity to inhibit the cytochrome metabolism.[17] Similarly, studies on Cocktail inhibition assays for assessing the drug-drug, drug-botanical interactions and assessing the six major cytochrome P450 enzymes was conducted by Guannan and Jing Wan respectively.[18, 19]

Drug–drug interactions may be identified during drug development and approval, food–drug interaction and supplements–drug interaction should not be overlooked. Natural health products are being widely used. Many plant products are useful in diabetes mellitus, anxiety, cancer and many such health problems.[20–23] Apart from an appraisal of product safety and effectiveness, attention should be paid to the potential that these product ingredients may interact with medications. In recent years, dietary carotenoids, especially xanthophylls, have attracted significant attention because of their activities as antioxidants and their roles in preventing cancer and age-related macular degeneration. Although around 40 carotenoids are ingested through a typical diet, only a few xanthophylls have been found in human tissues. There are few reports of inducible effects on CYP activities by the xanthophylls astaxanthin, b-cryptoxanthin, canthaxanthin,lutein, and zeaxanthin. However, their inhibitory effects on CYPs have rarely been investigated.[5]

CONCLUSION

Lutein obtained from marigold petals is of major commercial interest because of its use in functional food and cosmetics, as well as in pharmaceuticals. In conclusion, lutein extracted from Marigold exhibited a dose dependent inhibitory effect on CYP3A4. Results from this study provide information indicating the possibilities of herb-drug interaction if this extract is co-administered with the prescribed drugs that are metabo-

lized by CYP3A4. Further *in vivo* study is needed to investigate whether these effects are clinically significant.

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CONFLICT OF INTEREST

Nil

ABBREVIATIONS

JECFA - The Joint FAO/WHO Expert Committee on Food Additives

RSC - Royal Society of Chemistry

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GRAPHICAL ABSTRACT

SUMMARY

- Lutein was extracted from the flowers of *Tagetes erecta* L (Marigold) flowers family *Compositae*
- Lutein is chemically 3R, 3'R, 6'R-βε-carotene-3, 3'-diol
- All the tested concentrations (5 – 100µg/ml) of lutein showed dose – dependent manner inhibitory effect on Cytochrome P450 (CYP3A4) enzyme
- The IC_{50} value of lutein for CYP3A4 inhibitory activity was found to be 35.27µg/ml.
- Results indicate possibilities of herb-drug interaction if this extract is co-administered with the prescribed drugs that are metabolized by CYP3A4.

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