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CYTOCHROME P_{450} INHIBITION STUDY OF *PICRORHIZA KURROA*: EVALUATION OF HERB-DRUG INTERACTION

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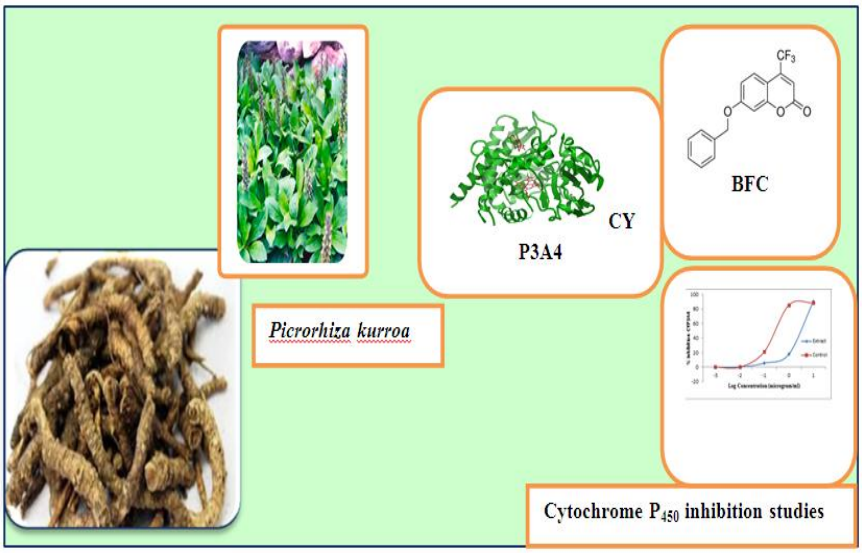

ABSTRACT

Background: *Picrorhiza kurroa* is an important medicinal plant in the traditional Chinese and Ayurvedic systems of medicine with wide therapeutic potential varing from anti-bacterial to immune-modulating properties. **Objective:** To establish the safety profile of *Picrorhiza kurroa* as a drug. **Material and Methods:** Cytochrome P_{450} mediated inhibitory potential is a useful tool to estimate the risk for drug interaction. To assess safety profile of *Picrorhiza kurroa*, CYP3A4 isoform was selected. Hydroalcoholic extract of *Picrorhiza kurroa* rhizome was prepared and assayed using a fluorescent substrate and recombinant CYP3A4 isoform. **Results:** It was observed that there is a very little possibility of herb-drug interaction of *Picrorhiza kurroa*, if it is administered along with other products that are metabolized by P_{450}. **Conclusion:** *Picrorhiza kurroa* is completely safe and can be used for therapeutic purposes. **Abbreviations used:** CYP, Cytochrome P_{450}; IC_{50}, Inhibitory concentration at 50%; BFC, 7-benzyloxy-4(trifluoromethyl)Coumarin.

KEYWORDS: *Picrorhiza kurroa*, Cytochrome P_{450} Inhibition, Herb-Drug interaction.

INTRODUCTION

Picrorhiza kurroa (Kutki, Kutka; Family Scrophulariaceae) is a perennial herb, abundantly found in the Himalayas. It is a significantly important plant in the traditional Chinese and Ayurvedic systems of medicine. It is reported to possess anti-bacterial, anti-periodic, hepatoprotective, anti-cholestatic[1],[2],[3],[4], anti-inflammatory, anti-oxidant[5],[6],[7], anti-allergy, anti-asthmatic[8],[9] and immune-modulating properties.[10] Due to immense therapeutic application of *Picrorhiza kurroa* in Ayurvedic medicine system it is necessary to evaluate its potential drug interaction with Cytochrome enzymes. The Cytochrome P_{450} isoenzymes (CYP) have an important role in xenobiotic metabolism and are involved in several types of interactions such as drugs, herbs, foods etc. Generally, the drug interactions involving CYP isoforms affects activation or inhibition of the enzyme.[11] The CYP mediated inhibitory activity is a useful tool to assess the risk for drug-drug interaction as it categories the compounds as high (IC_{50} <1 µM), moderate (1 µM<IC_{50}<10 µM) and low (IC_{50}>10 µM) potential for drug-drug interaction (DDI).

For the assessment of herb-drug interaction of *Picrorhiza kurroa*, CYP3A4 isoform (EC 1.14.13.97) was selected. Numerous other members of cytochrome P_{450} family are also involved in drug metabolism, but CYP3A4 is the most common and the most adaptable one. The use of CYP3A4 Inhibitor can decrease metabolism during the first pass and the elimination phases. Decreased drug elimination may enhance drugs therapeutic and also its toxicity. Thus, present research aims to develop the safety profile of *Picrorhiza kurroa* as a drug which will definitely be advantageous in endorsing traditional Ayurveda to the international level.

MATERIAL AND METHODS

Material

The rhizome of *Picrorhiza kurroa* was a generous gift from Patanjali Ayurveda Ltd., Haridwar. Recombinant CYP3A4 was purchased from XenoTech, LLC. Fluorescent substrate 7-benzyloxy-4(trifluoromethyl)Coumarin, CYP3A4 inhibitor Ketoconozole, solvents and chemicals were acquired from Sigma Aldrich, India.

Sample preparation

The *Picrorhiza kurroa* rhizomes were shade dried for nearly 3 weeks and coarse powder was prepared using a mixer grinder. The powdered, dried rhizome was extracted using petroleum ether (40-50^{o}C) for 18 h to remove the fat and other undesirable components. The resulted mixture was further treated with hydroalcoholic solution (60:40) for 18h and the extract was evaporated to dryness in a rotary flash evaporator at 60°C.

Assay Procedure

The CYP3A4 substrate (BFC) and the test compound were dissolved in 30% acetonitrile to prepare a stock solution of 10 mM. The assay was conducted using final volume of 200 µl, in a Microplate Fluorescence reader (BioTek FLx 800 T, U.S.A.). Cofactor (1.3 mM NADP, 3.3 mM glucose-6-phosphate, 3.3 mM $MgCl_2$ and 0.4 U/ml, glucose-6-phosphate dehydrogenase) was prepared using 100 mM of potassium phosphate buffer (pH 7.4). CYP3A4 enzyme was diluted to yield 1.5 pmole/well. The enzyme and cofactor were incubated in shaking incubator for 20 minutes at 37^{o}C. Substrate (50 µM final concentration), reference compound and test compound of each concentration were added in a 96 well black plate and incubated at 37^{o}C in a shaking incubator for 15 minutes. Incubation was continued for another 20 minutes with the addition of 100 µl of pre-warmed enzyme cofactor mix to the assay plate and the reaction was stopped by the addition of 50 µl of 50% acetonitrile and 20% Tris Base (0.5 M). Fluorescence per well was measured using an excitation wavelength of 410 nm and emission wavelength of 530 nm in the Fluorescence Reader. The data was analyzed using a Graph Pad Prism Software Version-5. The IC_{50} values were calculated using a Sigmoidal Dose Response.

Calculations and Statistical analysis

The obtained data was presented as mean ± S.E.M. The statistical significance was calculated by ANOVA using GraphPad InStat Version 5.0. In addition, the Dunnett's multiple comparison test was achieved by setting the significance level at $p<0.05$ and above.

Percent Inhibition calculated using below mentioned formula:

% Inhibition at x conc. = 100 -% Control

Where x denotes the inhibitor concentration, the IC_{50} value calculated using per cent inhibition data.

RESULTS AND DISCUSSION

Picrorhiza kurroa is metabolized by CYP3A4 as it has been shown to markedly reduce the clearance of fluorescence substrate of CYP3A4. A dose response study was conducted for *Picrorhiza kurroa*, using five concentrations (0.001, 0.01, 0.1, 1 and 10 µg/ml). Upto 0.01 µg/ml, the tests compound did not show any inhibition however, at higher concentrations the inhibition was prominent and showed a reduction in P_{450} concentration. Maximum inhibition of 90.6% was observed at 10 µg/ml of the test compound. The resulted inhibitory concentration at 50% (IC_{50}) of *Picrorhiza kurroa* was calculated to be 52.35µg/ml whereas for the inhibitor Ketoconozole, IC_{50} was 4.74 µg/ml. (**Figure 1**)

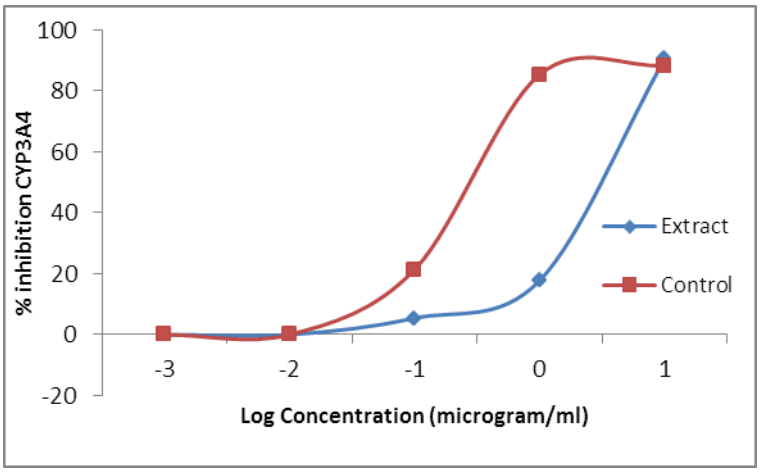

Figure 1. Percentage inhibitory effect of *Picrorhiza kurroa* extract and Ketoconazole on CYP3A4

The results indicate that the test extract has a higher IC_{50} value than respective positive inhibitor against CYP3A4. Higher IC_{50} value indicates that a high concentration of extract is required to reduce the total enzyme activity to its half (50%). The greater IC_{50} of the plant extract is desirable for the safety of the drug as it is less likely to display substantial drug interaction.

CONCLUSION

In the present study, we established the safety profile of *Picrorhiza kurroa* rhizome extract. It can be concluded that there is a very little possibility of herb-drug interaction of *Picrorhiza kurroa*, if it is administered along with other products that are metabolized by cytochrome P_{450}. The results confirmed that *Picrorhiza kurroa* can be used safely in the Ayurvedic formulations. However, advanced study is required to find out whether these inhibitory effects are clinically significant or not. Additionally, *in vivo* interactions must be taken into

account for better correlation between the potency and selectivity of cytochrome P_{450} inhibition.

Conflict of Interest

All authors declare that they have no conflict of interest.

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