ORIGINAL ARTICLES

IN VITRO INHIBITIONS OF CYP2C9 AND CYP3A4 BY FRACTIONS ISOLATED FROM GOJI BERRY (LYCIUM BARBARUM) FRUITS

Kaloyan D. Georgiev¹, Maya Radeva-Ilieva²

¹Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University of Varna
²Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University of Varna

ABSTRACT

INTRODUCTION: Lycium barbarum L., known as Goji berry, is widely used as a medicinal and functional food. Apart from the positive therapeutic properties it possesses, it can inhibit enzymes involved in the metabolism of drugs and it can cause herb-drug interactions (HDIs).

AIM: The aim of the study is evaluation of the potential of polysaccharide and polyphenolic fractions isolated from L. barbarum fruits to inhibit the activity of CYP3A4 and CYP2C9.

MATERIALS AND METHODS: The inhibitory effects of polysaccharide and polyphenolic fractions isolated from L. barbarum on CYP3A4 and CYP2C9 activity were determined using Vivid® CYP3A4 and CYP2C9 Green Screening Kits.

RESULTS: Both fractions inhibit the activity of investigated CYP isoenzymes. The polyphenolic fraction was more potent with IC₅₀ values of 0.119 mg/mL and 0.048 mg/mL against CYP3A4 and CYP2C9, respectively. The polysaccharide fraction has inhibited CYP3A4 and CYP2C9 with IC₅₀ values of 2.244 mg/mL and 4.094 mg/mL, respectively.

CONCLUSION: The use of beverages based on L. barbarum can lead to herb-drug interactions (HDIs). Further research in this direction will assess the significance of these interactions.

Keywords: Lycium barbarum, Goji berry, cytochrome P450 (CYPs), herb-drug interactions (HDIs)

INTRODUCTION

Lycium barbarum L. (Solanaceae), known as Goji berry, has been used in southeastern Asian countries for many years, as a medicinal and functional food. Nowadays, it is gaining popularity in Western countries because of the powerful antioxidant properties it possesses, contributing to anti-aging, antitumor activity and many other described positive properties (1-4). The main part of this plant
that is used is the fruit. *L. barbarum* polysaccharides (LBPs) are the most studied components in the fruits and are referred to as the primary for inducing pharmacological effects (5-7). Other components, such as flavonoids, phenolic acids, anthocyanins, carotenoids, etc., also contribute to the pharmacological effects of *L. barbarum* fruits.

Cytochrome P450 (CYP) plays an important role in the biotransformation of xenobiotics, including the most used therapeutic drugs. The CYP3A4 and CYP2C9 isoenzymes take part in the metabolism of nearly 50-60% of the marketed drugs, making them susceptible to develop pharmacokinetic drug interactions (8). Herbal medicines are nowadays widely used by patients claiming to be effective agents with little or negligible undesirable effects. Many of them, however, are likely to affect cytochrome enzymes, inhibit or induce them, and could lead to important clinical herb-drug interactions (HDIs) (9-11). It is therefore necessary to study these plant products in detail to assess their potential for interactions.

**AIM**

The aim of the present study is to evaluate the potential of polysaccharide and polyphenolic fractions isolated from *L. barbarum* fruits to inhibit the activity of CYP3A4 and CYP2C9.

**MATERIALS AND METHODS**

**Plant Material and Fractions**

*L. barbarum* fruits (Lot №: L05042017) were provided by Paula Fruits Ltd – an official importer of Goji berries for Bulgaria with guaranteed Chinese origin. The preparation of the polysaccharide and polyphenolic extracts used in the study and their analysis is described in detail in our previous article (12). All chemicals used for extraction and analysis were of analytical grade and were purchased from the local representatives of Merck (Darmstadt, Germany) and Sigma (St. Louis, USA), unless otherwise indicated.

**Determination of CYP3A4 and CYP2C9 Activity with Vivid® P450 Assay Kits**

The inhibitory effects of polysaccharide and polyphenolic fractions isolated from *Lycium barbarum* on CYP3A4 and CYP2C9 activity were determined using Vivid® CYP3A4 Green Screening Kit and Vivid® CYP2C9 Green Screening Kit following manufacturer’s instruction (13). Stock solutions (2.0 mg/mL) were prepared from both used fractions and the following concentrations were obtained by dilution: 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL. The samples in each well were mixed with a master pre-mix, comprising reaction buffer, CYP450 BACULOSOMES® (CYP3A4 or CYP2C9) reagent and regeneration system, which contained glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The mixture was incubated at room temperature for 20 min. We performed a pre-read of the fluorescence at this point to determine if there were fluorescent compounds in the polysaccharide and polyphenolic fractions and no fluorescence was detected. Following incubation, CYP enzyme-specific substrate di(benzyloxymethoxy)fluorescein (DBOMF, Vivid green substrate for CYP3A4) or benzylxy-methyl-fluorescein (BOMF, Vivid green substrate for CYP2C9) and NADP+ were added and the mixture was incubated at room temperature for 30 min. The reaction was stopped by the addition of 10 mcM ketoconazole (CYP3A4) or 30 mcM Sulfaphenazole (CYP2C9) and enzyme activity was evaluated by measuring the fluorescence at excitation/emission wavelength of 485/528 nm (BioTek Synergy 2). To determine reaction kinetics, we performed the Kinetic Measurement Protocol (described in the manufacturer’s instruction (13)), where the fluorescence was measured at 5 min intervals for 30 min.

**Statistical Analysis**

To calculate the percentage of inhibition, we used the following equation: Percentage of inhibition = 100 − ([Signal of well (RFU, relative fluorescence units) − Blank]/[Solvent control − Blank] ×100). To build the graphs and to calculate IC_{50} with a 95% confidence interval, we used four-parameter logistic curve (4PL) generated by the GraphPad Prism version 6.0 (GraphPad Software, USA). For extrapolation of the results in logarithmic manner, we used Microsoft Excel 2010.

**RESULTS**

Both isolated fractions from *L. barbarum* were tested for inhibition on CYP3A4 and CYP2C9.
In Vitro Inhibitions of CYP2C9 and CYP3A4 by Fractions Isolated from Goji Berry (Lycium Barbarum) Fruits

Assessment of CYP3A4 Inhibition by L. barbarum Fractions

To assess the inhibition of CYP3A4, we applied the polysaccharide and polyphenolic fractions from L. barbarum in following concentrations – 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL. Results are shown in Fig. 1.

The polysaccharide fraction did not show a strong inhibitory effect on the CYP3A4 isoenzyme. In the highest used concentration (2.0 mg/mL) 40% of the activity of the isoenzyme was inhibited. The polyphenolic fraction has showed a potent inhibition on CYP3A4. The highest concentration (2.0 mg/mL) inhibits 90% of the activity of the isoenzyme.

Assessment of CYP2C9 Inhibition by L. barbarum Fractions

The fractions isolated from L. barbarum were also tested for inhibition of CYP2C9 in the same concentration manner. The results on CYP2C9 were similar to those on CYP3A4 (Fig. 2).

The polysaccharide fraction has shown negligible inhibition on CYP2C9, while the polyphenolic fraction exhibits a significant inhibition of the isoenzyme activity.

Determination of IC₅₀ Values

In order to determine the approximate IC₅₀ values with 95% confidence interval (CI) of the individual fractions on the CYP3A4 and CYP2C9 isoenzymes, we have extrapolated the results in logarithmic manner. The results are summarized in Table 1.

As can be seen from the table, the polyphenol fraction is significantly more potent than the polysaccharide in terms of the inhibition of CYP3A4 and CYP2C9, ~ 20 folds and 85 folds, respectively.

DISCUSSION

A “superfruit” or “superfood”, as Lycium barbarum L. (Goji berry) is known in the Western countries, has many useful nutritional and medicinal properties such as antioxidant, antidiabetic, antihyperlipidemic, immunomodulatory, anticancer, neuroprotective etc. (14, 15). Despite all the beneficial effects of consumption of L. barbarum fruits, there are many reports that announce for serious drug interactions with enzymes involved in the first phase of drug metabolism.

Our results indicate that L. barbarum extracts have inhibitory effects on investigated CYPs – CYP3A4 and CYP2C9. Furthermore, the polyphenolic fraction has more a pronounced effect than the polysaccharide fraction (Table 1). This would presumably lead to possible herb-drug interactions (HDIs). These results are confirmed by other authors. Liu et al.
et al. (16) investigated in vitro juice, water and ethanol extracts of *L. barbarum* against major human phase I metabolism enzymes and proves significant inhibition. They also find that mechanism of inhibition is rather competitive than mechanism-based. In our study, the conducted kinetic model demonstrated shifting the curves to the right, which suggests excluding mechanism-based inhibition. We observed a concentration-dependent inhibition of isozymes, suggesting that the mechanism is competitive.

There are several published studies that demonstrate the significance of possible interactions of *L. barbarum* in clinical conditions (17-19). All three cited studies described interactions between *L. barbarum* and warfarin. Warfarin is from the group of anticoagulants, with a narrow therapeutic index and its more active enantiomer, S-warfarin, is metabolized by CYP2C9 (20). In all three cases, patients have consumed drinks based on *L. barbarum* – wine, juice or herbal tea, concomitant with warfarin and increased INR values were recorded. They have concluded that the interaction is associated with inhibition of warfarin metabolism and have recommended caution when consuming beverages containing *L. barbarum* simultaneously with warfarin.

**CONCLUSION**

In this study we demonstrated the potential of *L. barbarum* to inhibit two isoenzymes – CYP3A4 and CYP2C9, which play major role in the biotransformation of the drugs. We have also shown that polyphenolic components are more potent inhibitors of the isoenzymes than polysaccharide. The usage of beverages based on *L. barbarum* can lead to HDIs. Further research in this direction will assess the significance of these interactions.

**Acknowledgements**

This study was funded by Project 16021 ”Investigation of drug interactions on biotransformation level”, Science Fund of Medical University “Prof. Dr. Paraskev Stoyanov”.

**REFERENCES**


In Vitro Inhibitions of CYP2C9 and CYP3A4 by Fractions Isolated from Goji Berry (Lycium Barbarum) Fruits


