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

Kaloyan D. Georgiev[1], Maya Radeva-Ilieva[2]

[1]Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University of Varna
[2]Department 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 IC_{50} 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 IC_{50} 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)*

Address for correspondence:
Kaloyan Georgiev
Faculty of Pharmacy
Medical University of Varna
84 Tzar Osvoboditel Blvd
9000 Varna
e-mail: kaloyan.georgiev@mu-varna.bg

Received: April 5, 2019
Accepted: June 27, 2019

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 of 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 benzyloxy-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.

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.

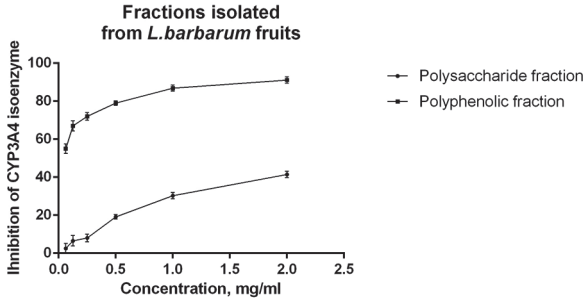

Fig. 1. Inhibition of CYP3A4 isoenzyme by polysaccharide and polyphenolic fractions isolated from L. barbarum fruits

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 IC_{50} Values

In order to determine the approximate IC_{50} 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.

Table 1. IC_{50} values with 95% confidence interval of the fractions isolated from L. barbarum on the activity of the CYP3A4 and CYP2C9 isoenzymes

	IC_{50} values (95% CI) on CYP3A4	IC_{50} values (95% CI) on CYP2C9
Polysaccharide fraction	2.244 mg/ml (1.593 to 3.161)	4.094 mg/ml (2.723 to 6.158)
Polyphenolic fraction	0.119 mg/ml (0.086 to 0.166)	0.048 mg/ml (0.036 to 0.063)

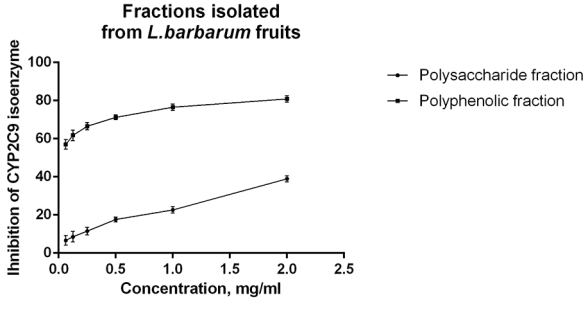

Fig. 2. Inhibition of CYP2C9 isoenzyme by polysaccharide and polyphenolic fractions isolated from L. barbarum fruits

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 R

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

1. Li QY. Healthy functions and medicinal prescriptions of Lycium barbarum (Gou Ji Zi). Beijing: Jindun Press; 2001. pp. 1–16.
2. Gong H, Shen P, Jin L, Xing C, Tang F. Therapeutic effects of Lycium barbarum (Goji) polysaccharide (LBP) on irradiation or chemotherapy-induced myelosuppressive mice. Cancer Biother Radiopharm 2005;20(2):155–62. doi: 10.1089/cbr.2005.20.155.
3. Gao Y, Wei Y, Wang Y, Gao F, Chen Z. Lycium Barbarum: A Traditional Chinese Herb and A Promising Anti-Aging Agent. Aging Dis. 2017;8(6):778–791. doi:10.14336/AD.2017.0725
4. Lee SR, Hwang HJ, Yoon JG, Bae EY, Goo KS, Cho SJ, Cho JA. Anti-inflammatory effect of Lycium barbarum on polarized human intestinal epithelial cells. Nutr Res Pract. 2019;13(2):95-104. doi: 10.4162/nrp.2019.13.2.95.
5. Cheng J, Zhou ZW, Sheng HP, He LJ, Fan XW, He ZX, Sun T, Zhang X, Zhao RJ, Gu L, Cao C, Zhou SF. An evidence-based update on the pharmacological activities and possible molecular targets of Lycium barbarum polysaccharides. Drug Des Devel Ther. 2014;9:33-78. doi: 10.2147/DDDT.S72892.
6. Masci A, Carradori S, Casadei MA, Paolicelli P, Petralito S, Ragno R, Cesa S. Lycium barbarum polysaccharides: Extraction, purification, structural characterisation and evidence about hypoglycaemic and hypolipidaemic effects. A review. Food Chem. 2018;254:377-389. doi: 10.1016/j.foodchem.2018.01.176.
7. Georgiev K, Jelev I, Georgieva S. Investigation of active ingredients of Goji berry (Lycium barbarum). Varna Medical Forum. 2013; 2(Suppl 3): 229-33.
8. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions substrates inducers and inhibitors. Drug Metab Rev. 1997;29(1-2):413–580.
9. Showande SJ, Fakeye TO, Kajula M, Hokkanen J, Tolonen A. Potential inhibition of major human cytochrome P450 isoenzymes by selected tropical medicinal herbs-Implication for herb-drug interactions. Food Sci Nutr. 2018;7(1):44–55. doi:10.1002/fsn3.789.
10. Fasinu PS, Bouic PJ, Rosenkranz B. The inhibitory activity of the extracts of popular medicinal herbs on CYP1A2, 2C9, 2C19 and 3A4 and the implications for herb-drug interaction. Afr J Tradit Complement Altern Med. 2014;11(4):54–61. doi:10.4314/ajtcam.v11i4.9.
11. Zhou S, Gao Y, Jiang W, Huang M, Xu A, Paxton JW. Interactions of herbs with cytochrome P450. Drug Metab Rev. 2003;35(1):35-98.

12. Georgiev KD, Slavov IJ, Iliev IA. Antioxidant activity and antiproliferative effects of Lycium barbarum's (Goji berry) fractions on breast cancer cell lines. Folia Med (Plovdiv) 2019;61(1): 104-12. doi: 10.2478/fomed-2018-0053.

13. Invitrogen. Vivid® CYP450 Screening Kits Protocol. 2005. http://ulab360com/files/prod/manuals/037/337004pdf. Accessed 10 Dec 2018

14. Jin M, Huang Q, Zhao K, Shang P. Biological activities and potential health benefit effects of polysaccharides isolated from Lycium barbarum L. Int J Biol Macromol. 2013;54:16-23. doi: 10.1016/j.ijbiomac.2012.11.023.

15. Georgiev KD, Slavov IJ, Iliev IA. Synergistic growth inhibitory effects of Lycium barbarum (Goji berry) extract with doxorubicin against human breast cancer cells. J Pharm Pharmacol Res 3(2019):051-058. doi: 10.26502/jppr.0020

16. Liu R, Tam TW, Mao J, Salem A, Arnason JT, Krantis A, Foster BC. In vitro activity of Lycium barbarum (Goji) against major human phase I metabolism enzymes. J Complement Integr Med. 2016;13(3):257-265. doi: 10.1515/jcim-2015-0038.

17. Zhang J, Tian L, Xie B. Bleeding due to a probable interaction between warfarin and Gouqizi (Lycium Barbarum L.). Toxicol Rep. 2015;2:1209-12. doi: 10.1016/j.toxrep.2015.08.011.

18. Rivera CA, Ferro CL, Bursua AJ, Gerber BS. Probable interaction between Lycium barbarum (goji) and warfarin. Pharmacotherapy. 2012;32(3):e50-3. doi: 10.1002/j.1875-9114.2012.01018.x.

19. Leung H, Hung A, Hui AC, Chan TY. Warfarin overdose due to the possible effects of Lycium barbarum L. Food Chem Toxicol. 2008;46(5):1860-2. doi: 10.1016/j.fct.2008.01.008.

20. Yamazaki H, Shimada T. Human liver cytochrome P450 enzymes involved in the 7-hydroxylation of R- and S-warfarin enantiomers. Biochem Pharmacol 1997;54:1195-203.