Effect of Myricetin on CYP2C8 Inhibition to Assess the Likelihood of Drug Interaction Using In Silico, In Vitro, and In Vivo Approaches

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ABSTRACT: Myricetin, a bioflavonoid, is widely used as functional food/complementary medicine and has promising multifaceted pharmacological actions against therapeutically validated anticancer targets. On the other hand, CYP2C8 is not only crucial for alteration in the pharmacokinetics of drugs to cause drug interaction but also unequivocally important for the metabolism of endogenous substances like the formation of epoxyeicosatrienoic acids (EETs), which are considered as signaling molecules against hallmarks of cancer. However, there is hardly any information known to date about the effect of myricetin on CYP2C8 inhibition and, subsequently, the CYP2C8-mediated drug interaction potential of myricetin at the preclinical/clinical level. We aimed here to explore the CYP2C8 inhibitory potential of myricetin using in silico, in vitro, and in vivo investigations. In the in vitro study, myricetin showed a substantial effect on CYP2C8 inhibition in human liver microsomes using CYP2C8-catalyzed amodiaquine-N-deethylation as an index reaction. Considering the Lineweaver−Burk plot, the Dixon plot, and the higher α-value, myricetin is found to be a mixed type of CYP2C8 inhibitor. Moreover, in vitro−in vivo extrapolation data suggest that myricetin is likely to cause drug interaction at the hepatic level. The molecular docking study depicted a strong interaction between myricetin and the active site of the human CYP2C8 enzyme. Moreover, myricetin caused considerable elevation in the oral exposure of amodiaquine as a CYP2C8 substrate via a slowdown of amodiaquine clearance in the rat model. Overall, the potent action of myricetin on CYP2C8 inhibition indicates that there is a need for further exploration to avoid drug interaction-mediated precipitation of obvious adverse effects as well as to optimize anticancer therapy.

1. INTRODUCTION

Myricetin (Figure 1) is one of the widely used bioflavonoids. It is predominantly present in various fruits and vegetables.\(^1,2\) It has diverse biological activities that are beneficial for the treatment of several diseases and therefore used as complementary/alternative medicine.\(^3\) Contemporary research evidence using an overwhelming number of in vitro/in vivo studies suggests that it is a promising anticancer candidate with impressive multifaceted pharmacological actions against therapeutically validated anticancer targets. Molecular targeting of cancer hallmarks by myricetin includes inflammation, cell proliferation, cell invasion, angiogenesis, metastasis, and apoptosis through activating/inactivating signaling pathways as well as reactivating tumor suppressor genes. Myricetin modulates the expression of various protein targets involved in cancer development such as mTOR, STAT3 (cell proliferation); CYCLIN B, p\(^{33}\) (cell cycle); VEGF, FOXM1 (angiogenesis); Bcl-2, BAX, caspase-3/8/9 (apoptosis); TNF-α, IFN-γ, COX-2, IL-1α (inflammation); N-CADHERIN, E-CADHERIN, MMP-1 (invasion and metastasis); and so on.\(^4\)

Cytochrome P450 (CYP) is mainly responsible for the metabolism of xenobiotics. Alteration in CYP levels by any candidate (drug/nutraceutical/function food) causes an increase or decrease in plasma concentration of the drug, leading to drug interaction (drug−drug/nutraceutical−drug/food−drug). This CYP-mediated pharmacokinetic interaction

Figure 1. Chemical structure of myricetin.

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may lead to precipitation of adverse effects or therapeutic failure of drugs unless otherwise devoid of any pharmacokinetic interaction, which is very beneficial for safe coadministration. Myricetin is reported to be able to interfere with several CYPs (CYP3A4/CYP2D6/CYP2C9/CYP2B6/CYP1A2) to modulate the pharmacokinetics of several drugs.5–7 However, there is hardly any information available on the CYP2C8 inhibitory potential of myricetin. This CYP2C8 is crucial for drug interaction and unequivocally important for the metabolism of endogenous substances like other key CYPs (CYP2E1, and CYP2J2). Basically, CYP2C8 participates in the oxidation of arachidonic acid into epoxyeicosatrienoic acids (EETs).8 These EETs act as signaling molecules and are implicated in tumor growth, angiogenesis, and suppression of inflammation.9 The proangiogenic role of EETs is quite clear and possibly support tumor growth via angiogenesis, but their role in cancer is still limited. Panigrahy et al. observed enhancement in the levels of EETs of endothelial cells, which caused tumor-associated angiogenesis and metastasis. Furthermore, the primary role of EETs is reported to cause primary growth of tumor due to angiogenesis, multiple organ metastasis, and lead the tumor to leave the dormant state.10 Moreover, it is evidenced that breast cancer is associated with augmented EET levels linked to upregulation of CYP2C8.11 Additionally, paclitaxel, an anticancer drug, is majorly metabolized by CYP3A4 and CYP2C8 in the liver and develops resistance toward malignant breast cancer cells, whereas the resistance can be overcome using carbon monoxide (a CYP2C8 inhibitor) in combination with paclitaxel.12 Therefore, targeting CYP2C8 inhibition can be very advantageous in cancer therapy, and an intended drug interaction may be beneficial toward improved efficacy and better tolerability of anticancer drugs.

In this pursuit, it is necessary to explore the CYP2C8 inhibitory potential of myricetin. Hence, we investigated the role of myricetin on CYP2C8 inhibition employing in silico, in vitro, and in vivo approaches.

2. RESULTS AND DISCUSSION

2.1. Effect on In Vitro CYP2C8 Inhibition in Human Liver Microsomes (HLMs). In the quest to explore any CYP2C8 inhibitory potential of myricetin, we first employed in vitro CYP2C8 inhibition studies. As per the USFDA guidelines, amodiaquine/amodiaquine-N-deethylation and paclitaxel/paclitaxel 6β-hydroxylation can be used as probe substrates/index reactions to study the enzymatic activity of CYP2C8.13,14 We chose amodiaquine/amodiaquine-N-deethylation over paclitaxel/paclitaxel 6β-hydroxylation considering the cost of substrate/metabolite. Therefore, the study was performed using amodiaquine-N-deethylation as an index reaction. At first, kinetic parameters for the index reaction were determined to ascertain the optimized conditions for evaluating the CYP2C8 inhibitory activity. N-Desethylamodiaquine as a metabolite was monitored in the reaction mixture and quantified by liquid chromatography tandem–mass spectrometry (LC–MS/MS) (Tables 1 and 2). The calculated maximum velocity of the uninhibited reaction (Vmax) and the Michaelis constant (Km) for the index reaction were 3996 ± 46 pmol/min/mg protein and 4.7 ± 0.12 μM, respectively (Figure 2A). The obtained results are in line with the reported values.15–17 After that, the concentration of amodiaquine (probe substrate) was fixed at 5 μM, which was close to its experimental Km value.13 Then, the IC50 of myricetin for CYP2C8 inhibitory action was estimated in HLM. The IC50 of quercetin as a positive control was assessed in parallel to the test compound and was found to be 2.8 ± 0.31 μM (Figure 2B), which corroborates the reported values for CYP2C8-catalyzed amodiaquine-N-deethylation.17,18 The experimental IC50 for myricetin was found to be 2.4 ± 0.05 μM (Figure 2C). Results demonstrate the substantial potential of myricetin to cause CYP2C8 inhibition in HLM. A potential inhibitor of CYP2C8, gemfibrozil is known to inhibit the enzymatic activity of CYP2C8 with IC50 values ranging from 91 to 120 μM. However, on glucuronidation, the IC50 value of gemfibrozil reduces to 24 μM.19,20 Gemfibrozil increased the oral exposure of cerivastatin (substrate of CYP2C8 and CYP3A4) in human subjects by about 5.6-fold.13

![Image]

**Table 1.** LC Conditions for Quantification of Amodiaquine and N-Desethylamodiaquine in In Vitro/In Vivo Experimental Samples

| Parameter                  | Condition                      |
|----------------------------|--------------------------------|
| Column                     | Purospher STAR RP-8 (125 x 2 mm, 5 μm) |
| Elution                    | Isoocratic                      |
| Mobile phase (% v/v)       | 0.1% formic acid in water: acetonitrile:: 60:40 |
| Flow rate                  | 0.3 mL/min                      |
| Column temperature (°C)    | 30°C                            |
| Autosampler temperature    | 4°C                             |
| Injection volume           | 2 μL                            |
| Retention time of amodiaquine | 0.4 min                      |
| Retention time of N-desethylamodiaquine | 0.4 min |
| Retention time of IS       | 0.9 min                         |
| Run time                   | 2 min                           |

![Image]

**Table 2.** MS/MS Conditions for Quantification of Amodiaquine and N-Desethylamodiaquine in In Vitro/In Vivo Experimental Samples

| Parameter                  | Value                              |
|----------------------------|-----------------------------------|
| Scan type                  | Selected reaction monitoring (SRM) |
| Source                     | Heated-electrospray ionization (H-ESI) |
| Ion polarity               | Positive                          |
| Vaporizer temperature (°C) | 300                               |
| Ion transfer tube temperature (°C) | 250                           |
| Sheath gas (arbitrary scale) | 30                               |
| Auxiliary gas (arbitrary scale) | 10                               |
| CID gas (mTorr)            | 1.5                               |
| Dwell time (ms)            | 148                               |
| RF lens for amodiaquine (V) | 154                              |
| RF lens for N-desethylamodiaquine (V) | 160                            |
| RF lens for IS (V)         | 58                                |
| Q1 resolution (FWHM)       | 0.7                               |
| Q3 resolution (FWHM)       | 0.7                               |
| Collision energy for amodiaquine (V) | 18                               |
| Collision energy for N-desethylamodiaquine (V) | 17                          |
| Collision energy for IS (V) | 20                               |
| Ion transition for amodiaquine (m/z) | 356.2 → 283.1                 |
| Ion transition for N-desethylamodiaquine (m/z) | 328.2 → 283.1                 |
| Ion transition for IS (m/z) | 180.0 → 110.1                   |
The next phase of experiments was performed to investigate the mode of inhibition for CYP2C8 by myricetin. To execute this study, the experimental $K_m$ value of amodiaquine ($\sim$5 $\mu$M) and the IC$_{50}$ value of myricetin ($\sim$2.4 $\mu$M) were used to select the range of concentrations for the probe substrate and the test candidate. This mechanistic study was done by varying the concentration of amodiaquine from 2.5 to 20 $\mu$M and myricetin from 1.2 to 9.6 $\mu$M. Myricetin exhibited a mixed type of inhibition, and the obtained $K_i$ value was 1.7 ± 0.11 $\mu$M with an $R^2$ value of 0.982. Based on the x-intercepts, y-intercepts, and slopes for different experimental data sets using visual inspection of the Lineweaver–Burk plot (Figure 3A), results did not match any model of three classical enzyme inhibition types: competitive, noncompetitive, and uncompetitive. Then, data were further evaluated using graphical analysis of the constructed Dixon plot (Figure 3B). Results of the mode of inhibition suggest typical diagnostic signatures of mixed-type inhibition. Two bioflavonoids, fisetin (IC$_{50}$ of 10.8 $\mu$M) and morin (IC$_{50}$ of 17.3 $\mu$M), also have CYP2C8 inhibitory activity via a mixed type of inhibition.

This mixed inhibition pattern can be observed when CYP enzymes metabolize substrates using different active sites or inhibitors block multiple active sites of CYP enzymes and disrupt enzyme activities. A mixed inhibitor interferes with the activity of an enzyme by binding to either the enzyme–substrate complex or the free enzyme; however, the affinity for both is different. The strength of binding energy between the enzyme and substrate alters in the presence of the inhibitor and is represented in terms of the $\alpha$-value. From the present research work, the obtained $\alpha$-value is 3.55, i.e., $>1$. This shows that myricetin might bind with the free CYP2C8 enzyme with a high affinity rather than with the enzyme–substrate complex, thus preventing the substrate’s binding with the enzyme. The competitive nature of inhibition by myricetin can also be linked to the relationship between $K_i$ and IC$_{50}$ of the inhibitor. For competitive, noncompetitive, and mixed inhibitors, $K_i = IC_{50}/2$, $K_i = IC_{50}$, and $K_i = IC_{50}/2$ to IC$_{50}$, respectively. From the in vitro results, the IC$_{50}$ and $K_i$ values obtained for myricetin were 2.4 and 1.7 $\mu$M, respectively. Thus, it is clear that myricetin is not a noncompetitive inhibitor but a mixed inhibitor that resembles competitive inhibition toward CYP2C8. Further study needs to be done to ensure myricetin interaction with CYP2C8 enzymes.

2.2. In Vitro–In Vivo Extrapolation for Prediction of Drug Interaction in Humans. From the in vitro results, it was found that myricetin can significantly inhibit the enzymatic activity of CYP2C8 in HLM. This data was then used to predict myricetin’s in vivo drug interaction potential in humans at the hepatic level. The maximum plasma concentration ($C_{max}$) of myricetin in plasma is reported to be 21.6 ± 5.7 $\mu$g/mL, i.e., $\sim$67874 nM, after oral administration of cranberry juice containing myricetin. A low concentration of micromolar protein was used in in vitro studies; therefore, nonspecific micromolar binding was considered negligible. The R-value illustrates the ratio of area under the curve (AUC) in the presence and absence of the inhibitor, which is directly proportional to $[1]/K_i$. Here, the calculated R-value is 40.9, i.e., $>1$. Therefore, results for in vitro to in vivo extrapolation reveal that myricetin has the potential to cause severe drug interaction at the hepatic level with drugs that are substrates of CYP2C8. Although in vitro—in vivo extrapolation results showed that myricetin could cause drug interaction at the
clinical level, ascertaining these prediction results can only be possible through clinical validation. Additionally, information of predicted results may be beneficial to avoid any drug interaction or may direct to take precaution during clinical exploration for precipitation of dose-related adverse effects. Typical examples for correlation of predicted results with the clinical outcome are as follows: The $K_i$ value of gemfibrozil (a known CYP2C8 inhibitor) for CYP2C8 inhibition in HLM was 10.2 $\mu$M, and the $C_{\text{max}}$ value was 16.85 $\mu$g/mL after dosing at 600 mg twice daily in healthy human volunteers. Thus, the calculated $R$-value for prediction of the drug interaction potential of gemfibrozil at the hepatic level was 1.7, i.e., >1.1. These predicted in vivo results of gemfibrozil are also confirmed by experimental (in vivo) results in humans, where gemfibrozil enhanced the $C_{\text{max}}$ and AUC of repaglinide, a probe substrate of CYP2C8, by 2.4- and 8.3-fold, respectively. Also, the bioavailability of sitagliptin (metabolized by CYP3A4 and CYP2C8) was enhanced by 54% in humans in the presence of gemfibrozil. Other examples include trans-resveratrol and quercetin, which inhibit the activity of CYP3A4 by $K_i$ values of 1.5 and 4.12 $\mu$M, respectively. Clinical studies showed that when 2000 mg and 100 mg doses of resveratrol and quercetin were given to humans, $C_{\text{max}}$ values of 1274 ng/mL and 2310 ng/mL were achieved. The $R$-values calculated from the prediction result for trans-resveratrol and quercetin were 4.7 and 2.6, respectively. From clinical studies, resveratrol was found to decrease the clearance by 33% and enhance the $C_{\text{max}}$ and AUC of carbamazepine (CYP3A4 substrate) by 1.5- and 1.4-fold, respectively. However, single-dose treatment of quercetin did not cause any alteration in the $C_{\text{max}}$ value was 16.85 $\mu$g/mL after dosing at 600 mg twice daily in healthy human volunteers. 

To verify a conjecture of myricetin interaction pattern with human CYP2C8 enzymes as well as before going into any in vivo validation for the CYP2C8 inhibitory potential of myricetin, we employed a molecular docking study to understand the favorable binding orientation, significant interactions, interacting amino acid residues, involved forces, binding affinity, and interaction energy between the active site of human CYP2C8 as the target enzyme and myricetin as the ligand. A rigid-based docking approach was applied with the help of the CDOCKER using a grid-based protocol in Biovia discovery studio 4.1 client software platform to assess the receptor–ligand interaction. The study was performed by selecting the best complex structure found as the best pose among all conformers of myricetin with the lowest CDOCKER interaction energy and CDocker energy of $-40.788$ kcal/mol and $42.618$ kcal/mol, respectively. Results showed that myricetin could interact with the active site of the human CYP2C8 enzyme. These interactions occurred through a hydrogen-bonding interaction with the amino acid residues like ASP A: 293, SER A: 114, GLY A: 98, and ARG A: 97 at distances of approximately 2.08, 1.84, 3.08, 2.24, and 2.70 Å, respectively; the $\pi–\pi$ bond interaction with amino acid residues like ILE A: 113, VAL A: 296, and PRO A: 367 at distances of approximately 4.63, 4.99, and 4.81 Å, respectively; the $\pi–\text{lone} \text{pair}$ bond interaction with the amino acid residue SER A: 103 at the distance of approximately 2.90 Å; and an attractive charge interaction with the amino acid residue ARG A: 97 at the distance of approximately 5.32 Å. Results illustrate that myricetin interacted with the active site of the human CYP2C8 enzyme primarily through ASP A: 293, ILE A: 113, VAL A: 296, SER A: 103, PRO A: 367, GLY A: 98, ARG A: 97, and SER A: 114 amino acid residues (Figure 4) with interacting forces like a conventional hydrogen bond, a $\pi–\text{hydrogen}$ bond, a $\pi–\text{lone}$ pair, attractive charge, and $\pi–\text{alkyl}$ bonding. Consequently, hydrogen bonding and $\pi–\text{hydrophobic}$ interactions with amino acid residues can identify the active binding site of the target protein in the complex structure. The above investigation determined that myricetin interacts with a higher number of active amino acid residues present in the active cavity of the enzyme with favorable interacting forces.

**2.4. Effect on In Vivo Pharmacokinetics of the CYP2C8 Substrate in a Rat Model.** On the basis of in silico, in vitro, and in vivo extrapolation results, it is evident that myricetin has CYP2C8 inhibitory potential to cause drug interaction in humans. Before going into dangerous and pricey clinical exploration, we thought that supportive discovery studio 4.1 client software platform to assess the receptor–ligand interaction. The study was performed by selecting the best complex structure found as the best pose among all conformers of myricetin with the lowest CDOCKER interaction energy and CDocker energy of $-40.788$ kcal/mol and $42.618$ kcal/mol, respectively. Results showed that myricetin could interact with the active site of the human CYP2C8 enzyme. These interactions occurred through a hydrogen-bonding interaction with the amino acid residues like ASP A: 293, SER A: 114, GLY A: 98, and ARG A: 97 at distances of approximately 2.08, 1.84, 3.08, 2.24, and 2.70 Å, respectively; the $\pi–\pi$ bond interaction with amino acid residues like ILE A: 113, VAL A: 296, and PRO A: 367 at distances of approximately 4.63, 4.99, and 4.81 Å, respectively; the $\pi–\text{lone}$ pair bond interaction with the amino acid residue SER A: 103 at the distance of approximately 2.90 Å; and an attractive charge interaction with the amino acid residue ARG A: 97 at the distance of approximately 5.32 Å. Results illustrate that myricetin interacted with the active site of the human CYP2C8 enzyme primarily through ASP A: 293, ILE A: 113, VAL A: 296, SER A: 103, PRO A: 367, GLY A: 98, ARG A: 97, and SER A: 114 amino acid residues (Figure 4) with interacting forces like a conventional hydrogen bond, a $\pi–\text{hydrogen}$ bond, a $\pi–\text{lone}$ pair, attractive charge, and $\pi–\text{alkyl}$ bonding. Consequently, hydrogen bonding and $\pi–\text{hydrophobic}$ interactions with amino acid residues can identify the active binding site of the target protein in the complex structure. The above investigation determined that myricetin interacts with a higher number of active amino acid residues present in the active cavity of the enzyme with favorable interacting forces.

![Figure 4](https://doi.org/10.1021/acsomega.2c00726)
model. In this context, all of the above-mentioned in vitro studies were executed in HLM, and therefore, we checked the functioning of the inhibitory effect of myricetin on amodiaquine metabolism using rat liver microsomes (RLMs). It was observed that the percent of amodiaquine remaining increased with the increasing concentration of myricetin (Supporting Information). This data indicates that myricetin could exhibit the inhibitory behavior on amodiaquine metabolism in RLM as well. Amodiaquine is metabolized majorly by CYP2C8 with minor contributions from CYP2D6 and CYP3A4. Myricetin is reported to inhibit the enzymatic activities of CYP3A4 (IC50 values of 10.7 or 20.3 μM) and CYP2D6 (IC50 value of 30.2 μM). Therefore, myricetin showed weak inhibition toward these two CYP isoforms, which suggest that any modulation in the pharmacokinetics of amodiaquine will be unlikely due to the interaction of myricetin with CYP3A4 or CYP2D6. Then, the pharmacokinetic study of amodiaquine was performed in the absence or presence of myricetin in rats. The present study aimed to determine the inhibitory effect of myricetin on the hepatic CYP2C8 enzyme. Therefore, myricetin was given to rats through the intravenous route to observe any inhibitory effect on the target enzyme using an in vivo model that resembles in vitro results. Amodiaquine, an antimalarial drug, is prescribed orally to patients, and thus, we decided to administer amodiaquine in rats through the oral route, which is common.

Figure 5. Representative SRM chromatograms for plasma sample analysis of pharmacokinetic studies in rats by LC-MS/MS: (A) blank plasma and (B) plasma sample spiked with amodiaquine in its SRM transition pair; (C) blank plasma and (D) plasma sample spiked with N-desethylamodiaquine in its SRM transition pair; and (E) blank plasma and (F) plasma sample spiked with phenacetin in its SRM transition pair.
for both treatment groups. The concentration of amodiaquine and its metabolite, N-desethylamodiaquine, in plasma was estimated by LC−MS/MS using a matrix match calibration standard. Method conditions were the same as described above (Tables 1 and 2), and representative chromatograms are depicted in Figure 5.

The mean plasma concentration versus time profiles of amodiaquine and N-desethylamodiaquine are represented in Figure 6. A similar observation for the biphasic behavior in the plasma exposure of amodiaquine is also reported at the preclinical level. The pharmacokinetic parameters were calculated on the basis of 60 mg/kg dose of amodiaquine and are summarized in Table 3. Myricetin resulted in an elevation of the overall plasma exposure of amodiaquine by 1.6-fold compared to amodiaquine alone ($p < 0.05$). Clearance of amodiaquine was reduced by 36% in the presence of myricetin in comparison to amodiaquine alone ($p < 0.05$). However, there was no considerable change in any other pharmacokinetic parameters of amodiaquine upon concomitant treatment of myricetin. Substantial elevation of plasma exposure of $N$-desethylamodiaquine was observed due to myricetin coadministration compared to amodiaquine administration without any myricetin treatment. The drug/metabolite ratio (amodiaquine/$N$-desethylamodiaquine) based on AUC $0-\infty$ was enhanced from 1.2 to 1.6 upon simultaneous coadministration of amodiaquine with myricetin. Similar observation during CYP-mediated delay in the metabolism of drugs, including amodiaquine, has been reported in the literature. Quercetin is reported to enhance the oral exposure of repaglinide by 1.8-fold in a rat model. Although amodiaquine has been used as a model drug in the present study, an alteration in the metabolism of such a drug can cause augmentation in oral exposure due to drug interaction, leading to precipitation of dose-dependent severe side effects like hepatotoxicity or agranulocytosis. To achieve the inhibitory effect of myricetin on the activity of hepatic

**Table 3. Pharmacokinetic Parameters of Amodiaquine and N-Desethylamodiaquine after Oral Administration of Amodiaquine (60 mg/kg) Alone (Group-I) and Intravenous Administration of Myricetin (5 mg/kg) Followed by Oral Administration of Amodiaquine (Group-II) in Rats**

| pharmacokinetic parameters | amodiaquine (group-I) | amodiaquine (group-II) | N-desethylamodiaquine (group-I) | N-desethylamodiaquine (group-II) |
|----------------------------|-----------------------|------------------------|-------------------------------|----------------------------------|
| $C_{\text{max}}$ (ng/mL)   | 1030.70 ± 246.80      | 1044.26 ± 121.61       | 370.40 ± 125.05               | 396.49 ± 32.00                  |
| $T_{\text{max}}$ (h)       | 3.80 ± 1.35           | 3.90 ± 1.29            | 4.20 ± 0.80                   | 3.60 ± 0.81                     |
| $AUC_{0-\infty}$ (ng·h/mL)| 3119.86 ± 414.44      | 4836.65 ± 608.37$^*$   | 1313.30 ± 349.54              | 2061.57± 452.16$^*$             |
| $T_{1/2}$ (h)              | 2.16 ± 0.30           | 2.49 ± 0.85            | 11.65 ± 3.21                  | 7.91 ± 3.00                     |
| $V_d/F$ (L/kg)             | 49.64 ± 11.02         | 33.92 ± 11.09          | 18.70 ± 4.68                  | 12.64 ± 4.42                    |
| $Cl/F$ (L/(h·kg))          | 15.67 ± 1.65          | 10.08 ± 1.36$^*$       | 11.65 ± 3.21                  | 7.91 ± 3.00                     |

$C_{\text{max}}$, maximum plasma concentration; $T_{\text{max}}$, time to reach $C_{\text{max}}$; $AUC_{0-\infty}$, AUC for plasma concentration from zero to the last measurable plasma sample time and to infinity; $T_{1/2}$, elimination half-life; MRT, mean residence time; $V_d/F$, volume of distribution after oral administration; and $Cl/F$, clearance after oral administration. Data are presented as mean ± SEM ($n = 5$). $^*p < 0.05$ denotes statistically significant when comparing Group-I versus Group-II.
CYP2C8 in rats, the dose of myricetin was given through intravenous route. The dose level of myricetin was chosen considering the fact of adequate plasma concentration to inhibit enzyme activity. The effect of myricetin upon any other route of administration can be performed, but the impact of physiological limitations should be taken into account if any. If we consider the consequences of drug interaction at the clinical level, then the pharmacokinetic interaction of repaglinide can be described as a suitable example for reference. Repaglinide is an oral hypoglycemic and is metabolized by CYP2C8 and CYP3A4. It is recommended as a clinical substrate drug for CYP2C8-mediated clinical drug interaction by USDA. Known CYP2C8 inhibitors like trimethoprim and clopidogrel elevated the oral exposure of repaglinide upon coadministration by 2-fold and 5-fold, respectively, which can lead to hypoglycemia in diabetic patients. Considering the potential of myricetin as a CYP2C8 inhibitor as well as an anticancer agent, further exploration should be done to observe its beneficial action via the intended drug interaction in cancer.

3. CONCLUSIONS

Integrating the above findings from \textit{in silico}, \textit{in vitro}, and \textit{in vivo} approaches, it can be concluded that myricetin has significant potential to cause pharmacokinetic interaction with a CYP2C8 substrate drug, and therefore, intake of myricetin as complementary medicine should be taken into account to optimize drug therapy. To the best of our knowledge, this is the first report of myricetin documenting the CYP2C8 inhibition-mediated drug interaction, which should be taken into account during concomitant therapy with CYP2C8 substrates. Considering the substantial anticancer potential of myricetin, further investigations on myricetin should be directed to establish the CYP2C8 inhibition-mediated drug interaction as a “friend” or “foe” under the purview of cancer treatment.

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents. Myricetin (≥96%), quercetin dihydrate (≥97%), phenacetin (≥98%), amodiaquine dihydrochloride dihydrate (≥97%), N-desethylamodiaquine dihydrochloride (≥95%), and nicotinamide adenine dinucleotide phosphate (NADPH) (>98%) were purchased from Sigma-Aldrich. HLM (pool of 50 donors; lot no# PL050D-A) and rat liver microsomes (RLM, lot no# RT053-3) were obtained from Gibco. Dimethylsulfoxide (DMSO) and PEG-400 were obtained from Loba Chemie. Acetonitrile, methanol, formic acid, and water (HPLC-grade and MS-grade) were procured from Thermo Fisher. The other chemicals or reagents were of bioreagent-grade or above.

4.2. \textit{In Vitro} CYP2C8 Inhibition in HLM. 4.2.1. Estimation of Kinetic Parameters. The study was performed in HLM using an index reaction of CYP2C8-catalyzed amodiaquine-N-deethylation. The stock solution of amodiaquine was prepared in methanol, and further dilutions were carried out using phosphate buffer (100 mM, pH 7.4). The dilution of N-desethylamodiaquine (supplied in methanol at 1 mg/mL) was done with methanol for preparation of standard solutions. The reaction mixture (200 μL) consisted of phosphate buffer (100 mM, pH 7.4), microsomal protein, i.e., HLM (0.025 mg/mL), MgCl₂ (3.3 mM), amodiaquine (0.25–100 μM), and NADPH (1.2 mM). The reaction was started by the addition of NADPH and incubated in a shaking water bath (37 °C, 20 min). After incubation, quenching of the reaction was done by placing the sample tubes in a thermal block and adding ice-cold acetonitrile (200 μL). Samples were vortex-mixed, centrifuged (3000 rpm, 15 min), decanted into inner vials, and quantitated by LC–MS/MS (make: Thermo Fisher Scientific; model: Ultimate 3000 for HPLC and TSQ-Endura for MS). The metabolite was measured in the reaction mixture using a matrix match protein standard (3.9–1000 ng/mL) by LC–MS/MS in the present and subsequent \textit{in vitro} experiments. Reactions were performed in triplicate. The organic content present in the reaction was limited to 0.5% (v/v). The reaction was optimized in such a way so that the maximum rate for the generation of metabolite was limited to less than 20%. The data of N-desethylamodiaquine was used to quantitate \( V_{\max} \) and \( K_{m} \).

4.2.2. Determination of \( IC_{50} \). The CYP2C8 inhibitory activities of the standard and test compounds in HLM were carried out using the optimized reaction condition except for the concentration of the probe substrate, which was close to the experimental \( K_{m} \) value. Quercetin and myricetin were used as the standard inhibitor (0.1–25 μM) and the test compound (1–50 μM), respectively. The reactions were performed in triplicate, and the reaction in the absence of a test compound was considered as a control. Quantitative data of N-desethylamodiaquine was used to calculate the \( IC_{50} \) value of the standard inhibitor and myricetin.

4.2.3. Assessment for the Mode of Inhibition. To assess the inhibition constant (\( K_{i} \)) and mechanism of inhibition by myricetin on CYP2C8, the chosen concentration levels for the present experimentation were at 0.5 \( \times \) \( K_{m} \), 2 \( \times \) \( K_{m} \), and 4 \( \times \) \( K_{m} \) for the probe substrate and 0, 1, 2, 4, 8, and 9.6 μM for myricetin. The rest of the reaction conditions were the same as mentioned above. The reaction was performed in triplicate. The obtained data were used to plot the Lineweaver–Burk plot and the Dixon plot.

4.2.4. Data Analysis. GraphPad Prism software was used to assess all of the parameters. The kinetic parameters (\( V_{\max} \) and \( K_{m} \)) for the CYP2C8 enzymatic activity in HLM were determined by fitting the data on the rate for metabolite formation versus probe substrate concentrations to nonlinear regression analysis. The \( IC_{50} \) values of the standard inhibitor and myricetin were estimated using data on the % control of CYP2C8 enzymatic activity after inhibition at a log of varied inhibitor concentrations. Data on the rate of metabolite generation at the various concentrations of probe substrate and myricetin were fitted to the different types of enzyme inhibition model to determine the apparent \( K_{i} \) and mode of CYP2C8 inhibition by myricetin.

4.3. \textit{In Vitro}–\textit{In Vivo} Extrapolation for Prediction of Drug Interaction in Humans. The \textit{in vitro} data of myricetin for CYP2C8 inhibition in HLM was used to predict the potential of drug interaction at the hepatic level. To calculate the R-value, we used our experimental \( K_{i} \) value and \( C_{\text{max}} \) of myricetin from the literature. Human subjects were given 237 mL of a double-strength (54% juice) cranberry juice cocktail, and myricetin content in the cranberry juice was a maximum of 47 mg/L. The calculation was done using the following equation

\[
R_{\text{value}} = \frac{\text{AUC}_{\text{I}}}{\text{AUC}_{\text{UI}}} = 1 + \frac{[I]}{K_{i}}
\]

where AUC\textsubscript{I} and AUC\textsubscript{UI} are the areas under the curve of test compound and control under identical conditions.
where AUC$_i$/AUC$_0$ is the ratio of AUC of the substrate in the presence of an inhibitor (AUC$_i$) to the AUC without an inhibitor (AUC$_0$), $K_i$ is the in vitro inhibition constant, and $[I]$ is the C$_{max}$ of myricetin for prediction at the hepatic level.

4.4. Effect on In Silico CYP2C8 Interaction in Humans.

A molecular docking study was carried out for a better understanding of the interaction pattern between the active site of the target enzyme (CYP2C8) and myricetin. The 3D structure of the enzyme (human CYP2C8) was retrieved from the Protein Databank (pdb id: 2NNI) and used in the software platform.38–40 Before molecular docking, energy minimization of both the enzyme and myricetin was performed using the CHARMM force field with the Momany–Rone partial charge method.53 Polar hydrogen atoms were included in the structure, and all ionizable residues were set to their default protonation state at neutral pH using the option “ligand and protein preparation” protocol instigated in the software platform. The active site of the enzyme was defined as the point where it contains active amino acid residues such as ARG A: 97*, SER A: 100, SER A: 103*, ARG A: 105, ILE A: 106*, THR A: 107*, ILE A: 112, ILE A: 113*, SER A: 114*, TRP A: 120, ARG A: 124, ARG A: 132, ASN A: 133, ARG A: 200, PHE A: 201, ASN A: 204, LEU A: 208, ILE A: 213, ASN A: 217*, PHE A: 226, PRO A: 227, GLY A: 228, THR A: 229, HIS A: 230, LEU A: 234, VAL A: 237, THR A: 240, LEU A: 249, VAL A: 296*,ALA A: 297, GLY A: 298, THR A: 301, THR A: 302, THR A: 305, VAL A: 366, HIS A: 368, PRO A: 427, PHE A: 428, SER A: 429, ARG A: 433, CYS A: 435, GLY A: 437, ALA A: 441, and VAL A: 477 in the active pocket of the enzyme.54 Then, the grid was prepared using different options in the software platform. During molecular docking, the translation center of the ligand was stimulated to a definite position inside the active site of the enzyme, formed a series of random spins, and generated random conformers of the molecules, where each conformer was thereafter relaxed by simulated annealing molecular dynamics.53 It basically involved heating (700 K in 2000 steps) and cooling (300 K in 5000 steps) the model candidate. The study was executed within a sphere with a radius of 27.1501 Å and XYZ coordinates of 51.092, 13.374, and −26.881 Å, respectively, in the active site of the enzyme. After molecular docking, generated poses were sorted according to the CHARMM energy, and the specific structure was carefully chosen according to the COCKER interaction energy for further analysis.

4.5. Effect on In Vivo Pharmacokinetics of the CYP2C8 Substrate. 4.5.1. Animal Model and Ethical Prerequisite. The study was performed in healthy male Wistar rats (8–10 weeks of age). Rats were maintained in typical laboratory conditions under a standard pellet diet and free access to water. Institutional Animal Ethics Committee of our institute (IAEC No: 228/78/2/21) gave the approval to execute the study.

4.5.2. Test Article and Dose Formulation. The present investigation was done using amodiaquine as a CYP2C8 substrate. The human dose of amodiaquine, i.e., 10 mg/kg/day, is converted to obtain the rat dose (62 ± 60 mg/kg).46 Based on the present literature information regarding pharmacokinetic studies in the presence of myricetin, the dose of myricetin was selected at 5 mg/kg.55 The vehicle was 1% DMSO + 19.5% PEG-400 + 79.5% water (v/v), which was used to prepare the individual dose formulation of amodiaquine and myricetin. The dose was prepared freshly and administered at 10 mL/kg (dose-volume).

4.5.3. Study Design. The study was performed using overnight fasted rats with water ad libitum and randomly divided into two groups on the day of experimentation. The first set of animals (Group-I, n = 5) was treated orally with amodiaquine alone. The second set of animals (Group-II, n = 5) was treated intravenously with myricetin followed by oral administration of amodiaquine. To achieve the maximum effect due to myricetin treatment, it was given 0.5 h before amodiaquine dosing. After amodiaquine administration, blood samples were collected in the microcentrifuge tube with an anticoagulant. Time points for blood sample collection (~120 µL) are as follows: 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 h. After that, centrifugation was done for 10 min at 8000 rpm to obtain the plasma sample (50 µL) and stored in a deep freezer (~ −80 °C) until analysis.

4.5.4. Bioanalysis. Individual stock solutions (1 mg/mL) of drug (amodiaquine), metabolite (N-desethylamodiaquine), and internal standard (phenacetin) were prepared or diluted further in methanol accordingly to obtain the desired concentration. The plasma protein precipitation technique was adopted for the processing of samples.22 An individual plasma sample (50 µL) was processed with the addition of acetonitrile (200 µL) containing an internal standard (200 ng/mL), vortex-mixed, and centrifuged (10 min, 14,000 rpm), decanted, and then poured into a vial for analysis by LC−MS/MS (Supporting Information). Matrix match calibration standards were prepared by spiking amodiaquine and N-desethylamodiaquine into blank plasma and processed as mentioned above. Data were processed by LCQUAN software. The LC−MS/MS system (HPLC model: Ultimate 3000; MS model: TSQ-Endura; make: Thermo Fisher Scientific) was used to quantitate amodiaquine and N-desethylamodiaquine in the processed samples of pharmacokinetic studies.

4.5.5. Pharmacokinetic Data Evaluation. Plasma concentration data was fitted into a noncompartmental model using PK solution software to calculate the following pharmacokinetic parameters (C$_{max}$ T$_{max}$ AUC$_{0−t}$ AUC$_{0−∞}$ T$_{1/2}$ MRT, V$_{d}$/F, and CI/F).

4.6. Statistical Analysis. Statistical data was analyzed using the unpaired Student’s t-test (GraphPad Prism), where p < 0.05 was considered statistically significant.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00726.

Methodology and results for the effect of myricetin on metabolic depletion of amodiaquine (PDF)

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Notes

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