# IMPACT OF HCA, QCN AND GLZ ON PHARMACOKINETICS OF QTE

# 6. Impact of HCA, QCN & GLZ on Pharmacokinetics of QTE

## 6.1. Preparation of Dosing Formulations

HCA formulation was prepared daily by dissolving it in saline (0.9% *w/v* sodium chloride in water) solution and mixing vigorously. To study pharmacokinetic interaction potential of HCA, its formulation was dosed at 20 mg/kg/day to rats, considering the dose volume of 10 mL/kg (groups I and III) for 7 days as mentioned in Section 3.12. The dose administered to rats was estimated based on the recommended human dose following US FDA guidelines [1].

Typical human dose of QCN supplements is 500-1000 mg per day (7-14 mg/kg for an average human weight of 70 kg). To study pharmacokinetic interaction potential of QCN, its formulation was dosed at 10 mg/kg to rats, considering the dose volume of 10 mL/kg (groups I and III) for 7 days through *p.o*. route as mentioned in Section 3.13. Similar dose has been reported in literature to study QCN interaction potential on pharmacokinetics of other drugs. QCN formulation was prepared daily by dissolving it in 20% *v/v* glycerol in normal saline (0.9% *w/v* sodium chloride in water) and mixing vigorously.

To study pharmacokinetic interaction potential of GLZ, its formulation was dosed at 100 mg/kg/day to rats, considering the dose volume of 10 mL/kg (groups I and III) for 7 days as mentioned in Section 3.14 through *p.o*. route. Similar dose has been reported in literature to study GLZ interaction potential on pharmacokinetics of other drugs. GLZ formulation was prepared daily by dissolving it in 20% *v/v* glycerol in normal saline (0.9% *w/v* sodium chloride in water) and mixing vigorously.

In case of QTE, dose of 1 mg/kg (*i.v.*) and 10 mg/kg (*p.o.*) was selected, as much lower dose could have resulted in systemic concentration less than limit of quantitation of the UPLC-MS/MS method due to high first pass metabolism of QTE. On the other hand, higher doses could have resulted in saturation of elimination pathways and hence non-linear pharmacokinetics. QTE was dissolved and diluted with normal saline (0.9% *w/v* sodium chloride in water).

## 6.2. Effects of HCA, QCN AND GLZ on the Metabolic Stability of QTE in RLS9

The effect of HCA or QCN on the metabolic stability of QTE was investigated by adding either HCA or QCN (final concentration of 10 μg/mL) to 40 μL of RLS9 and 712 μL of PBS buffer was added to 1.5 mL centrifuge tubes and pre-incubating them for 5 minutes at 37 °C (in the presence of NADPH). QTE was also incubated in the presence of itraconazole (100 ng/mL) as a positive control [2]. Solvent control was also incubated with QTE to understand the impact of time on enzyme stability.

The effect of GLZ on the metabolic stability of QTE was investigated by incubating QTE in RLS9 obtained from rats which were pre-treated with GLZ for seven days. Incubation was done as mentioned in above section of HCA or QCN. Testosterone was also incubated in RLS9 (control rats and GLZ pre-treated rats), which acted as a positive control.

After 5 minutes, 8 μL QTE solution (final concentration of 1 μg/mL) was added. Aliquots of 30 μL were collected from reaction volumes at 0, 5, 15, 30, & 60 minutes and 120 μL acetonitrile containing internal standard (100 ng/mL of Propranolol) was added to terminate the reaction and the concentration of the QTE was determined using UPLC-MS/MS. The slopes and *in vitro* half-life ($t_{1/2}$) were calculated as described in the previous section. QTE was also incubated in the presence of itraconazole (100 ng/mL) as a positive control.

### 6.3. Sample Processing

Sample extraction for HCA was performed using single-step protein precipitation with 10% *v/v* TCA in Milli-Q water (containing 500 ng/mL of FA). In brief, 30 μL of study/CS/QC samples were mixed with 150 μL of 10% *v/v* TCA in water and vortex mixed for 10 minutes followed by centrifugation (Eppendorf Centrifuge 5804 R, Germany) at 2755 ×g for 5 minutes at 4 °C. Supernatant solution (2 μL) was injected into the UPLC-MS/MS.

For QCN, GLZ and QTE, aliquots of 30 μL of rat plasma or brain homogenate were transferred into vials. Afterwards, acetonitrile (150 μL) containing internal standard (200 ng/mL of FA and propranolol each) was added to precipitate proteins. The mixture was vortex mixed for 5 minutes and centrifuged at 10000 rpm for 5 minutes at 4 °C. Each sample supernatant (150 μL) was filtered through a 0.22 μm syringe filter, and 2 μL aliquot of the filtered sample was injected into the UPLC-MS/MS system.

### 6.4. Instrumentation and Chromatographic Conditions

Chromatographic separation was performed on an Acquity UPLC™ (Waters Corporation, Milford, USA) consisting of an Acquity binary solvent manager and mass analysis was done on a 4000 MS/MS (Applied Biosystems, MDS Sciex, Canada).

Details of the bio-analytical method used for estimation of HCA are mentioned in Section 3.8. Briefly, Acquity HILIC column (150 × 2.1 mm, 1.7 μm, Waters Corporation, Milford, USA) maintained at 40 °C was used to retain HCA. Samples were kept at 15 ± 3 °C in auto-sampler. The mobile phase A consisted of 10 mM ammonium bicarbonate prepared in Milli-Q water and mobile phase B consisted of 100% acetonitrile. MRM mode was selected to capture transitions of HCA; Q1:Q3::206.8:127 and FA; Q1:Q3::280:236. Total run time was 5 minutes and the injection volume was 2 μL. Quadrupole Q1 and Q3 were set on the unit resolution.

Method details for estimation of QCN, GLZ, GA and QTE are mentioned in Section 3.9. Briefly, Kinetex C18 column (50 mm × 2.1 mm, 1.7 μm, Phenomenex, USA) maintained at 40 °C was used to retain QTE. Samples were kept at 15 ± 3 °C in auto-sampler. The mobile phase A consisted of 10 mM ammonium formate prepared in Milli-Q water and mobile phase B consisted of 0.1% *v/v* formic acid in acetonitrile. Total run time was 2.5 minutes and the injection volume was 2 μL. The turbo gas temperature was set at 550 °C. UPLC-MS/MS was run in polarity switching mode. MRM positive mode was selected to capture transitions of QTE; Q1:Q3::384.5 :253.4; GLZ; Q1:Q3::823.2:453.2 and Propranolol; Q1:Q3::260.2:183.2. MRM positive mode was selected to capture transitions of GA; Q1:Q3::469.2:425.2; QCN; Q1:Q3::301.2:150.9 and flufenamic acid; Q1:Q3::281.2:236.1. Quadrupole Q1 and Q3 were set on the unit resolution. For acquisition and chromatogram data processing, Analyst software version 1.6 (Applied Biosystems, Sciex Canada) was used.

## 6.5. Results and Discussion

### 6.5.1. Impact of HCA on Pharmacokinetics of QTE

Toxicological assessment of herbal extracts or natural products is extremely important mainly when these supplements are consumed along with allopathic medicines. Many scientists claim that interactions between herb and drugs are hypothetically more difficult to understand than usual DDIs (drug-drug interactions) due to the multi-constituent nature of herbal extracts or natural products. Nevertheless, the scarcity of systematic data about the clinical implications of drug interactions with herbal extracts or natural products makes it even more difficult in making informed choices during their co-administration. It is reported that co-administration of *G. cambogia* containing dietary supplement with serotonin reuptake inhibitor, escitalopram in a patient resulted in serotonin toxicity [3]. On the other hand, numerous case studies about instances of toxicity leading to psychosis or even death after the intake of dietary supplements containing *G. cambogia* are available in literature [4,5]. Chuah *et al.* (2012) reviewed clinical reports for efficacy and safety of HCA and *G. cambogia*-containing supplements and revealed that no adverse/toxic effects were seen up to 2800 mg/day of HCA consumption [6].

Commonly used anti-psychotic agent QTE has a narrow therapeutic window [7]. QTE interactions with co-administered drugs have been well recognized. Though, paltry evidence exists about QTE interactions with herbal extracts or natural products. Moreover, its primary route of elimination is through hepatic metabolism (fraction metabolized, fm > 0.85 through CYP3A4), which makes it vulnerable for drug-drug or drug-herb interactions. QTE's brain penetration is regulated through P-gp [7]. Recently, a human casualty has been reported due to QTE toxicity complicated by mitragynine, which is a major active ingredient of herb Kratom [8,9]. Mitragynine

has inhibitory effects on multiple cytochrome P450 enzymes including CYP2D6 (non-competitive), CYP2C9 (non-competitive), and CYP3A4 (competitive) [9]. Other than this singular report, no information is available about QTE interactions with any of the natural products used as dietary supplements.

### 6.5.1.1. *In vivo* Studies to Understand Impact of HCA on PK of QTE

The validated UPLC-MS/MS method was successfully used to quantify QTE in rat plasma and brain homogenate. The mean plasma concentration-time profiles for QTE after oral administration of QTE (with & without pre-treatment using HCA) are presented in Figure 6.1.

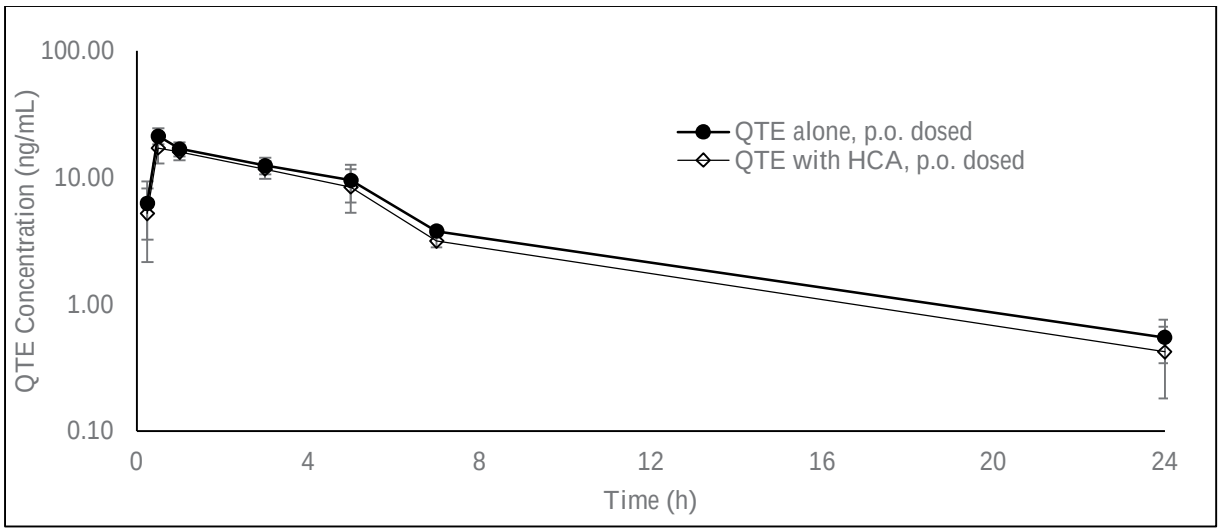



**Figure 6.1.** Plasma concentration-time profile of QTE after administration of QTE (*p.o.*) alone and along with HCA in Wistar rats (n=6).

The plasma concentration-time was analyzed using the non-compartmental analysis to determine pharmacokinetic parameters. Table 6.1 summarizes the pharmacokinetic parameters for QTE after *p.o.* administration of QTE alone and in combination with HCA.

**Table 6.1.** Pharmacokinetic parameters of QTE in Wistar rats (n=6) following *p.o.* administration of QTE alone and QTE along with HCA.

| PK Parameters | QTE along with HCA (group I) | QTE alone (group II) |
|---|---|---|
| $T_{max}$ (h) | 0.50 ± 0.00 | 0.50 ± 0.00 |
| $C_{max}$ (ng/mL) | 19.42 ± 2.48 | 21.24 ± 3.07 |
| $C_{last}/C_{max}$ | 0.04 ± 0.02 | 0.03 ± 0.01 |
| $AUC_{0\text{-}last}$ ( ng*h/mL) | 120.848 ± 17.50 | 114.691 ± 14.91 |
| $AUC_{0\text{-}inf}$ ( ng*h/mL) | 125.56 ± 16.74 | 118.43 ± 13.17 |
| $C_{last}$ (ng/mL) | 0.79 ± 0.19 | 0.55 ± 0.21 |
| Brain Concentration (ng/mL) | 1.75 ± 0.32 | 1.45 ± 0.40 |

BLQ: LLOQ for HCA: 10.5 ng/mL; Values expressed as Mean± SD

There was no impact on $C_{max}$ of QTE upon HCA pre-treatment (19.42 ± 2.48 *Vs.* 21.24 ± 3.07 ng/mL) and a similar trend was observed in $AUC_{(0\text{-last})}$ (120.848 ± 17.50 *Vs.* 114.691 ± 14.91 ng*h/mL). The brain to plasma ratio was 2.22 in rats exposed to pre-treatment with HCA and 2.64 for rats pre-treated with the vehicle. There was no change in HCA pharmacokinetic profile upon repeat administration of HCA for 7 days and also QTE co-administration did not significantly alter $C_{max}$ and AUC of HCA. The mean plasma concentration-time profiles for QTE after *i.v.* administration of QTE (with and without pre-treatment using HCA) are presented in Figure 6.2.

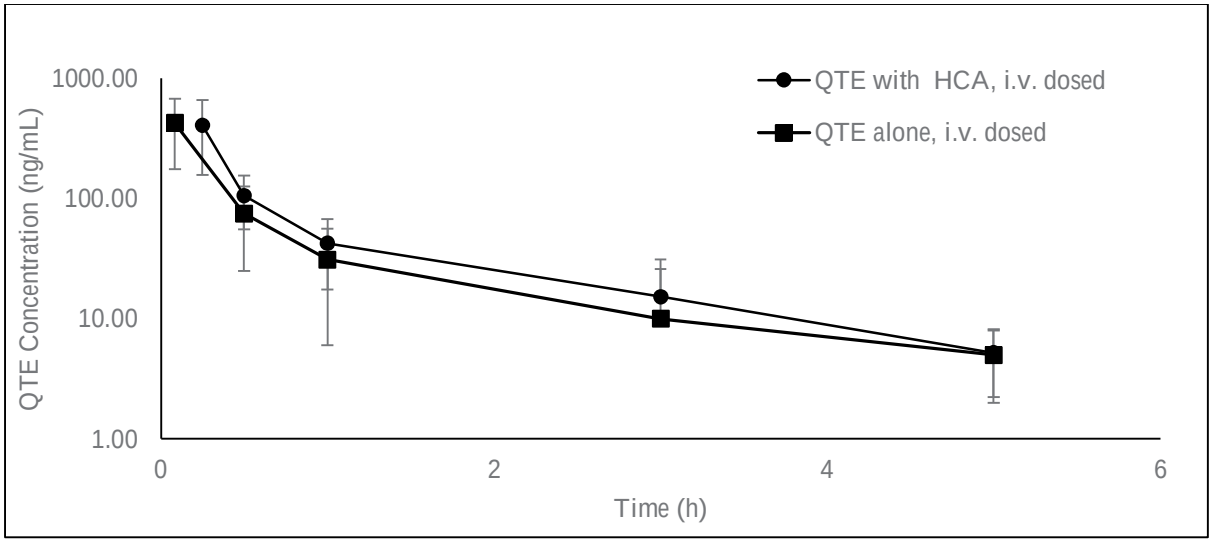



**Figure 6.2.** Plasma concentration-time profile of QTE after administration of QTE (*i.v.*) alone and along with HCA in Wistar rats (n=6).

The pharmacokinetic parameters of QTE and HCA after *i.v.* administration of QTE (1 mg/mL) (control with HCA pre-treatment) and pre-treated with HCA at 20 mg/kg for 7 days administration are compiled in Table 6.2.

**Table 6.2.** Pharmacokinetic parameters of QTE in Wistar rats (n=6) following *i.v.* administration of QTE alone and QTE along with HCA.

| **PK Parameters** | **QTE along with HCA (group III)** | **QTE (group IV)** |
|---|---|---|
| $C_0$ (ng/mL) | 556.84 ± 95.84 | 600.24 ± 126.51 |
| $AUC_{0\text{- last}}$ (ng*h/mL) | 248.15 ± 67.62 | 229.08 ± 75.94 |
| $AUC_{0\text{-inf}}$ (ng*h/mL) | 259.57 ± 75.41 | 240.26 ± 81.63 |
| Cl (mL/min/kg) | 69.51 ± 3.84 | 72.68 ± 4.07 |
| $V_{z\_obs}$ [(mg/kg)/(ng/ml)] | 0.0085 ± 0.0007 | 0.0091 ± 0.0006 |
| $t_{1/2}$ (h) | 1.49 ± 0.09 | 1.52 ± 0.08 |
| Brain Concentration (ng/mL) | BLQ | BLQ |

BLQ: Below limit of quantification (LLOQ; 0.1 ng/mL); Values expressed as Mean± SD

There was no impact on $C_{max}$ of QTE upon HCA pre-treatment (600.24 ± 126.51 *Vs* 556.84 ± 95.84 ng/mL) and a similar trend was observed in $AUC_{0\text{-last}}$ (229.08 ± 75.94 *Vs* 248.15± 67.62 ng*h/mL). The brain concentrations at 24 h were below limit of quantitation (LLOQ: 0.1 ng/mL) in rats from both groups (pre-treated with HCA and pre-treated with the vehicle).

#### 6.5.1.2. Effects of HCA on the Metabolic Stability of QTE in RLS9

QTE (1 μg/mL) was rapidly metabolised in RLS9 ($t_{1/2}$ = 5-10 minutes) and metabolized completely in 60 minutes. Thus, high hepatic clearance of QTE in *in vivo* might be expected.

Figure 6.3 exhibits QTE concentration when incubated in the presence of HCA. The post hoc test further revealed that in HCA (10 μg/mL) treated group, the trend of QTE metabolism were similar to without HCA incubation in the RLS9. Positive control worked as expected, wherein complete inhibition was observed in the presence of itraconazole (100 ng/mL).

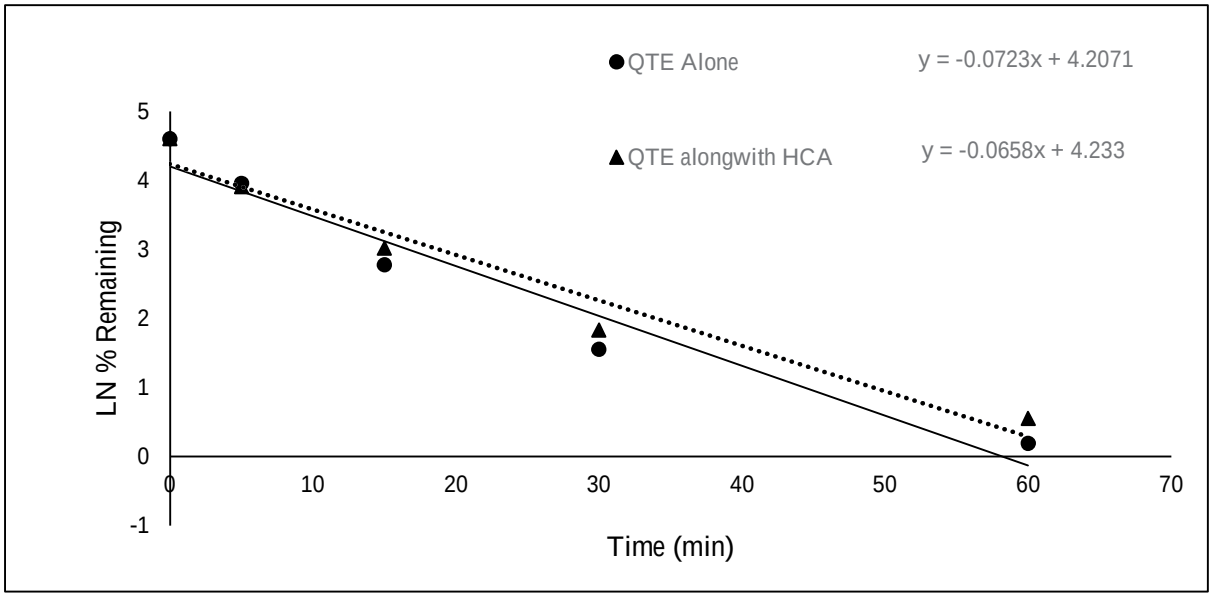



**Figure 6.3.** Semi-log plot of QTE remaining *Vs* time (minutes) incubated in RLS9 in the presence and absence of HCA.

The data found in the present study about the effects of HCA solution on the PK of QTE did not raise any alarms linked to the manifestation of significant natural product-drug interactions. The study describes that no statistically significant difference was found in the extent of plasma exposure (AUC), $C_{max}$, other PK parameters and subsequently the systemic bioavailability of QTE in the presence and absence of HCA. The HCA administration along with QTE is improbable to be clinically relevant. Since QTE is primarily eliminated through hepatic metabolism through CYP3A4 enzymes (fm > 0.85) and hence is prone to enzyme modulation and time-dependent inhibition/induction mechanisms of enzymes, the results suggest that HCA solution has no perceptible effects (inhibition/induction) on the pharmacokinetics of QTE.

The likely interference of anaesthetic agent (pentobarbital) was investigated during the method development and validation and no significant interference was observed for QTE analysis. The anaesthetic agent was used on all groups (experimental and control groups) of animals one day before QTE administration for insertion of a cannula in the jugular vein of rats. It is expected that anaesthetic agent was eliminated because all the rats had usual activity. No distinctive monitoring was performed and the animals had free access to diet in group III and IV. In group I and II, rats were overnight fasted with free access to water before QTE administration, and to avoid the interaction of food on systemic exposure of QTE, the fasting period was continued for 4 h after administration of QTE.

### 6.5.2. Impact of QCN on Pharmacokinetics of QTE

Our studies demonstrated 3 folds increase in peak to trough ratio for QTE in the presence of QCN, suggesting that the $C_{max}$ of the former is hitting very high when co-administered with QCN [10-13]. This concentration could be high enough to cross NOAEL (no observed adverse effect level), potentially leading to toxicity.

#### 6.5.2.1. *In vivo* Studies to Understand Impact of QCN on PK of QTE

The validated UPLC-MS/MS method was successfully used to quantify QTE in rat plasma and brain homogenate. The mean plasma concentration-time profiles for QTE after *p.o.* administration of QTE (with and without pre-treatment using QCN) & QTE after *i.v.* administration are presented in Figure 6.4.

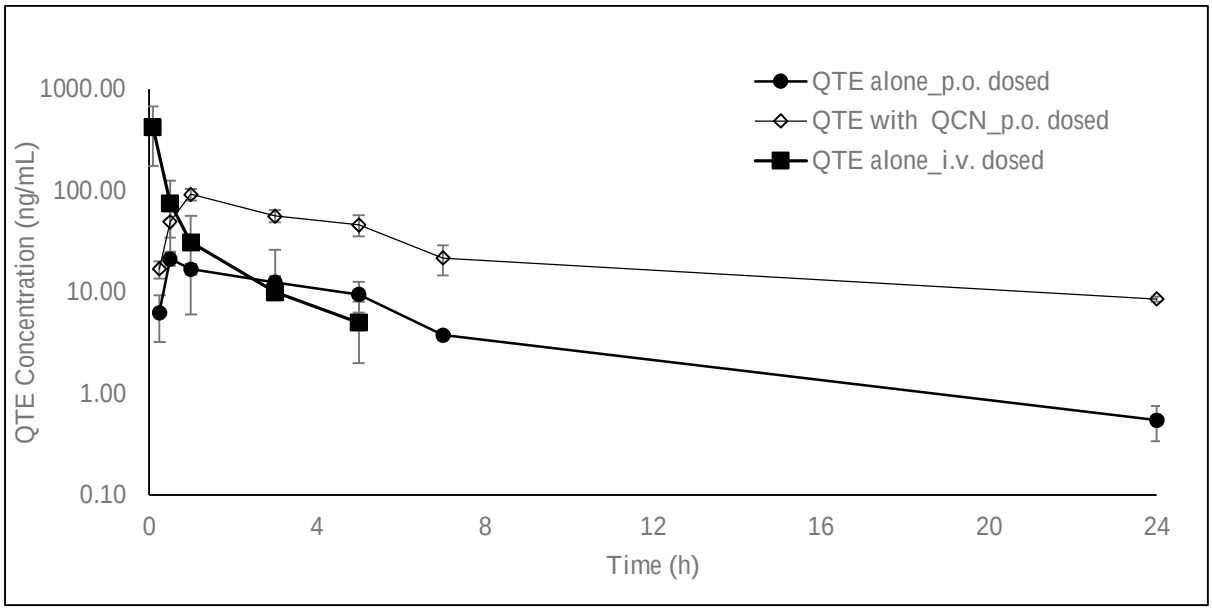



**Figure 6.4.** Plasma concentration-time profile of QTE after administration of QTE (*i.v.* and *p.o.*) alone and along with QCN (*p.o.*) in Wistar rats (n=6).

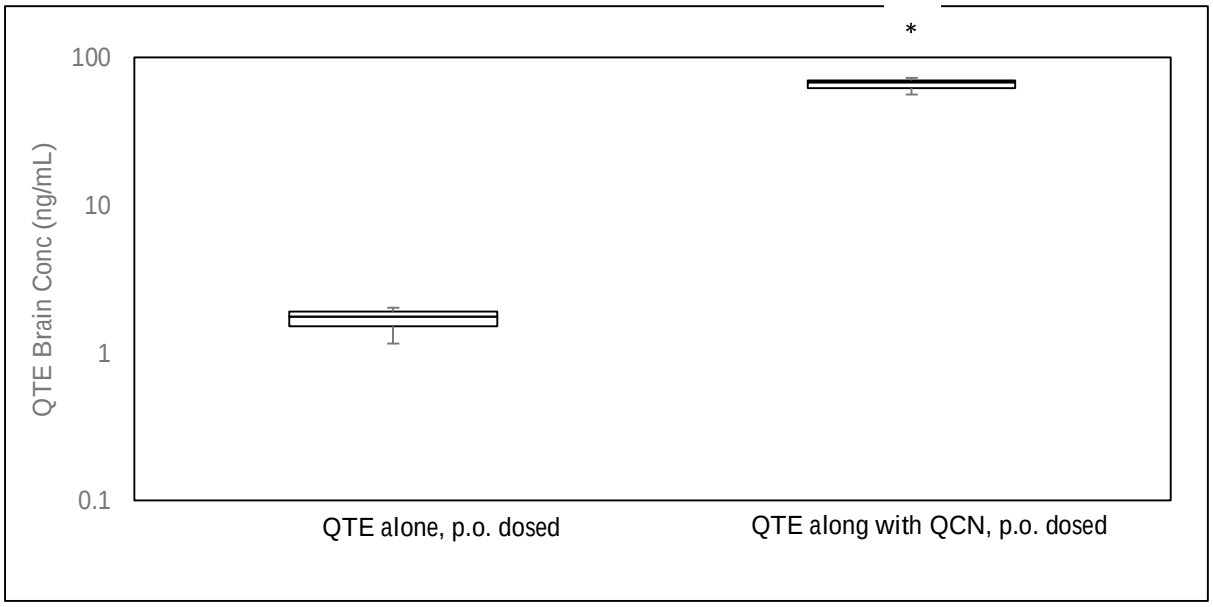
The plasma concentration-time was analysed using the non-compartmental analysis to determine pharmacokinetic parameters. Table 6.3 summarizes the pharmacokinetic parameters for QTE, after *p.o.* (with and without QCN) administration. The significant increase $C_{max}$ of QTE upon QCN pre-treatment (92.07 ± 12.15 *Vs.* 20.48 ± 4.91 ng/mL) and a similar trend was observed in $AUC_{(0\text{-last})}$ (627.98 ± 80.33 *Vs.* 120.64 ± 25.47 ng*h/mL).

**Table 6.3.** Pharmacokinetic parameters of QTE in Wistar rats (n=6) following *p.o.* administration of QTE alone and QTE along with QCN

| **PK Parameters** | **QTE along with QCN (group I)** | **QTE alone (group II)** |
|---|---|---|
| $T_{max}$ (h) | 1.00 ± 0.00 | 0.50 ± 0.00 |
| $C_{max}$ (ng/mL) | 92.07 ± 12.15 | 20.48 ± 4.91 |
| $C_{last}/C_{max}$ | 0.094 ± 0.02 | 0.02 ± 0.01 |
| $AUC_{0\text{-last}}$ (ng*h/mL) | 627.98 ± 80.33 | 120.64 ± 25.47 |
| $AUC_{0\text{-inf}}$ (ng*h/mL) | 723.78 ± 85.73 | 135.71 ± 15.03 |
| $C_{last}$ (ng/mL) | 8.57 ± 0.49 | 0.45 ± 0.16 |
| Brain Concentration (ng/mL) | 66.25 ± 11.83 | 1.37 ± 0.68 |

Values expressed as Mean± SD

The brain to plasma ratio was 7.73 in rats exposed to pre-treatment with QCN and 3.04 for rats pre-treated with vehicle. Box and Whisker plot for brain concentration (ng/mL) of QTE after dosing alone and along with QCN is presented in Figure 6.5. There was no change in QCN pharmacokinetic profile upon repeat administration of QCN for 7 days and also QTE co-administration did not significantly alter $C_{max}$ and AUC of QCN.




*p<0.05 indicates significant difference in QTE exposure upon pre-treatment with QCN.*

**Figure 6.5.** Box and Whisker plots for brain concentration (ng/mL) of QTE after dosing alone and along with QCN.

The pharmacokinetic parameters of QTE after *i.v.* administration of QTE (1 mg/mL) are compiled in Table 6.4. For group III (QCN pre-treated), all six rats died within 5 minutes of *i.v.* administration of QTE. The average brain concentration was 2 μg/mL in this group (group III).

**Table 6.4.** Pharmacokinetic parameters of QTE in Wistar rats (n=6) following *i.v.* administration of QTE alone and QTE along with QCN

| **PK Parameters** | **QTE (group III)** |
|---|---|
| $C_0$ (ng/mL) | 594.62 ± 134.06 |
| $AUC_{0\text{-last}}$ (ng*h/mL) | 249.61 ± 62.74 |
| $AUC_{0\text{-inf}}$ (ng*h/mL) | 273.92 ± 77.39 |
| Cl (mL/min/kg) | 73.06 ± 5.11 |
| $V_{z\_obs}$ [(mg/kg)/(ng/ml)] | 0.0072 ± 0.0005 |
| $t_{1/2}$ (h) | 1.63 ± 0.07 |
| Brain Concentration (ng/mL) | BLQ |

BLQ: Below limit of quantification (LLOQ; 0.1 ng/mL); Values expressed as Mean± SD

### 6.5.2.2. Effects of QCN on the Metabolic Stability of QTE in RLS9

Figure 6.6 exhibits QTE concentration when incubated in the presence of QCN. The post hoc test further revealed that in QCN (10 μg/mL) treated group, the levels of QTE metabolism were significantly reduced in the RLS9. The half-life was increased 10 folds (from 5 minutes to 50 minutes) due to inhibition of CYP3A4. Positive control worked as expected wherein complete inhibition was observed in the presence of itraconazole (100 ng/mL), suggesting that CYP3A4 was involved in the metabolism of QTE.

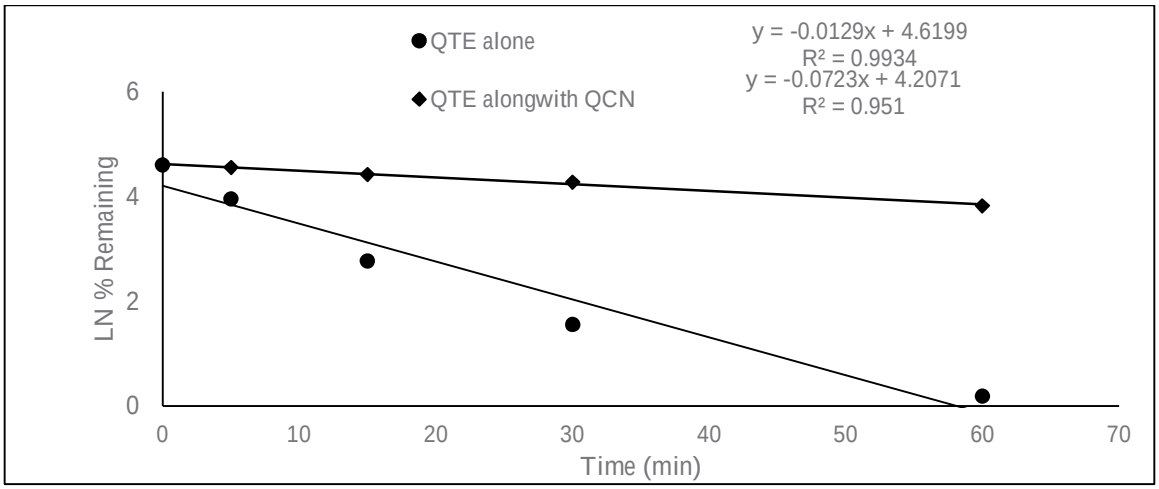



**Figure 6.6.** Semi-log plot of QTE remaining *Vs.* time (minutes) incubated in RLS9 in the presence and absence of QCN

### 6.5.3. Impact of GLZ on Pharmacokinetics of QTE

Numerous constituents of licorice are described to impact the activity of CYP3A4 enzyme [14-21]. QTE is metabolized via hepatic and intestinal CYP3A4 enzyme. The induction of CYP3A4 may reduce the systemic exposure of QTE. The nuclear receptors like PXR (pregnane X receptor)

and CAR (constitutive androstane receptor) control the expression of CYP3A4 and P-gp. PXR is modulated by various endogenous and/or exogenous components. It is described that natural products could regulate the activity of CYP3A4 and P-gp. For example, licorice at a dose of 3 g/kg have been reported to induce P450 levels by ~91% and is dose-dependent. The PXR activation by GLZ is also reported to induce the abundance of CYP3A4. Besides, few studies indicated that the major contributing component was GA, which is a major metabolite of GLZ and it induces CYP3A4 significantly.

### 6.5.3.1. *In vivo* Studies to Understand Impact of GLZ on PK of QTE

The validated UPLC-MS/MS method was successfully used to quantify QTE in rat plasma and brain homogenate. The mean plasma concentration-time profiles for QTE after *p.o.* administration of QTE (with and without pre-treatment using GLZ) are presented in Figure 6.7.

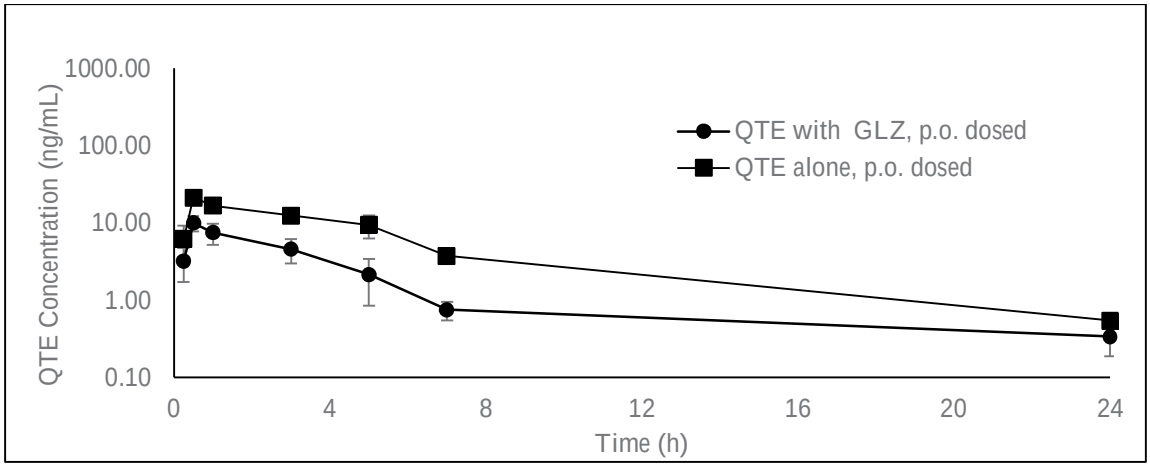



**Figure 6.7.** Plasma concentration-time profile of QTE after administration of QTE (*p.o.*) alone and along with GLZ in Wistar rats (n=6).

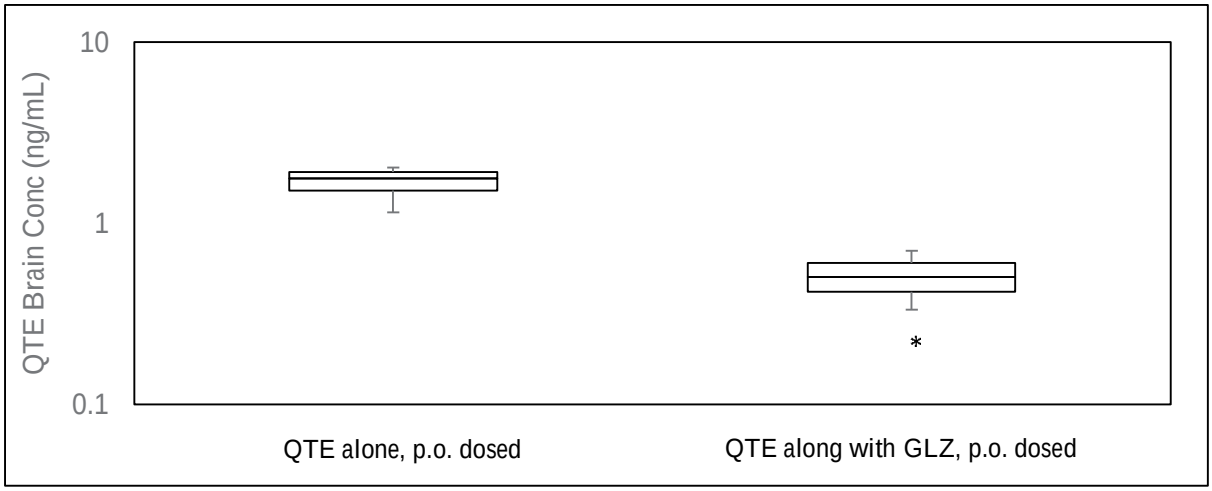
The plasma concentration-time was analyzed using the non-compartmental analysis to determine pharmacokinetic parameters. Table 6.5 summarizes the pharmacokinetic parameters for QTE, after *p.o.* (with and without GLZ) administration.

**Table 6.5.** Pharmacokinetic parameters of QTE in Wistar rats (n=6) following *p.o.* administration of QTE alone and QTE along with GLZ.

| PK Parameters | QTE along with GLZ (group I) | QTE alone (group II) |
|---|---|---|
| $T_{max}$ (h) | 1.00 ± 0.00 | 0.50 ± 0.00 |
| $C_{max}$ (ng/mL) | 10.05 ± 2.21 | 20.48 ± 4.91 |
| $C_{last}/C_{max}$ | 0.03 ± 0.01 | 0.02 ± 0.01 |
| $AUC_{0\text{-}last}$ (ng*h/mL) | 72.25 ± 18.57 | 120.64 ± 25.47 |
| $AUC_{0\text{-}inf}$ (ng*h/mL) | 78.51 ± 14.20 | 135.71 ± 15.03 |
| $C_{last}$ (ng/mL) | 0.34 ± 0.13 | 0.45 ± 0.16 |
| Brain Concentration (ng/mL) | 0.3 ± 0.07 | 1.37 ± 0.68 |

Values expressed as Mean± SD

As evident, the $C_{max}$ of QTE decreased significantly upon GLZ pre-treatment (10.05 ± 2.21 *Vs.* 20.48 ± 4.91 ng/mL) and a similar trend was observed in $AUC_{0\text{-}last}$ (72.25 ± 18.57 *Vs.* 120.64 ± 25.47 ng*h/mL). The brain to plasma ratio was 0.88 in rats exposed to pre-treatment with GLZ and 3.04 for rats pre-treated with vehicle. Box and Whisker plot for brain concentration (ng/mL) of QTE after dosing alone and along with GLZ is presented in Figure 6.8. There was no change in GLZ pharmacokinetic profile upon repeat administration of GLZ for 7 days and also QTE co-administration did not significantly alter $C_{max}$ and AUC of GLZ.




*p<0.05 indicates significant difference in QTE exposure upon pre-treatment with GLZ*

**Figure 6.8.** Box and Whisker plots for brain concentration (ng/mL) of QTE after dosing alone and along with GLZ.

The pharmacokinetic parameters of QTE after *i.v.* administration of QTE (1 mg/mL) are compiled in Table 6.6.

**Table 6.6.** Pharmacokinetic parameters of QTE in Wistar rats (n=6) following *i.v.* administration of QTE alone and QTE along with GLZ

| **PK Parameters** | **QTE along with GLZ (group III)** | **QTE (group IV)** |
|---|---|---|
| $C_0$ (ng/mL) | 324.17 ± 75.49 | 594.62 ± 134.06 |
| $AUC_{0\text{-}last}$ (ng*h/mL) | 125.41 ± 58.61 | 249.61 ± 62.74 |
| $AUC_{0\text{-}inf}$ (ng*h/mL) | 154.26 ± 42.84 | 273.92 ± 77.39 |
| Cl (mL/min/kg) | 135.52 ± 5.84 | 73.06 ± 5.11 |
| $V_{z\_obs}$ ((mg/kg)/(ng/ml)) | 0.0073 ± 0.0006 | 0.0072 ± 0.0005 |
| $t_{1/2}$ (h) | 0.67 ± 0.07 | 1.63 ± 0.07 |
| Brain Concentration (ng/mL) | BLQ | BLQ |

BLQ: Below limit of quantification (LLOQ; 0.1 ng/mL); Values expressed as Mean± SD

The mean plasma concentration-time profiles for QTE after *i.v.* administration of QTE (with and without pre-treatment using GLZ) are presented in Figure 6.9.

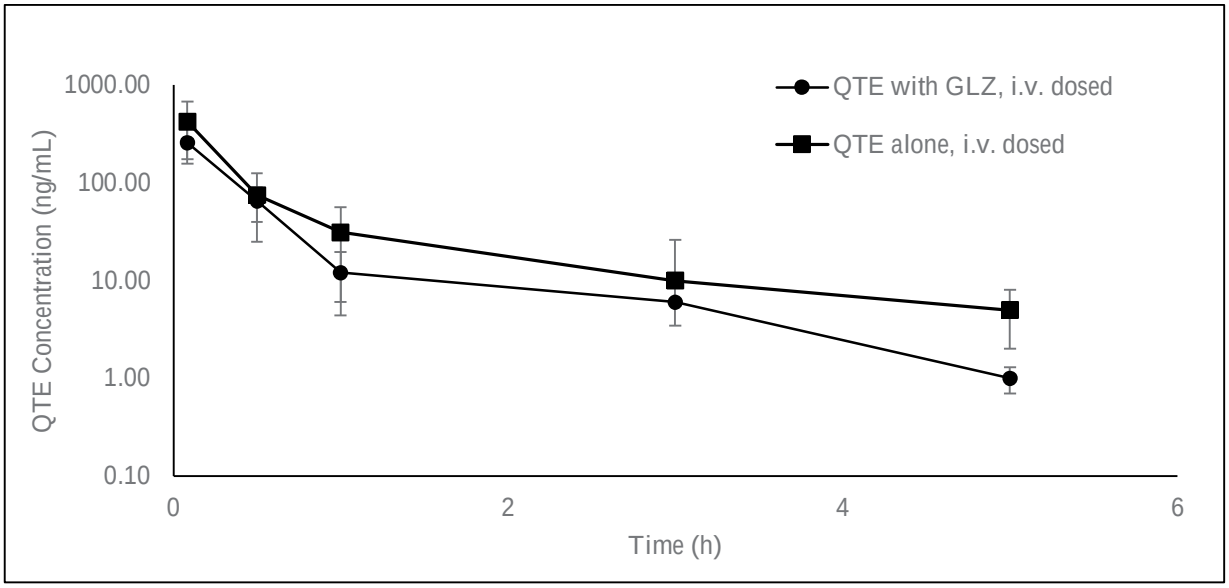



**Figure 6.9.** Plasma concentration-time profile of QTE after administration of QTE (*i.v.*) alone and along with GLZ in Wistar rats (n=6).

### 6.5.3.2. Effects of GLZ on Metabolic Stability of QTE in RLS9

QTE concentration after incubation in RLS9 isolated from the rats pre-treated with GLZ is depicted in Figure 6.10. The post hoc test further revealed that in RLS9 isolated from GLZ pre-treated group, the levels of QTE metabolism were significantly increased. The half-life was decreased 2.1 folds (from 9.5 minutes to 4.6 minutes) due to induction of CYP3A4.

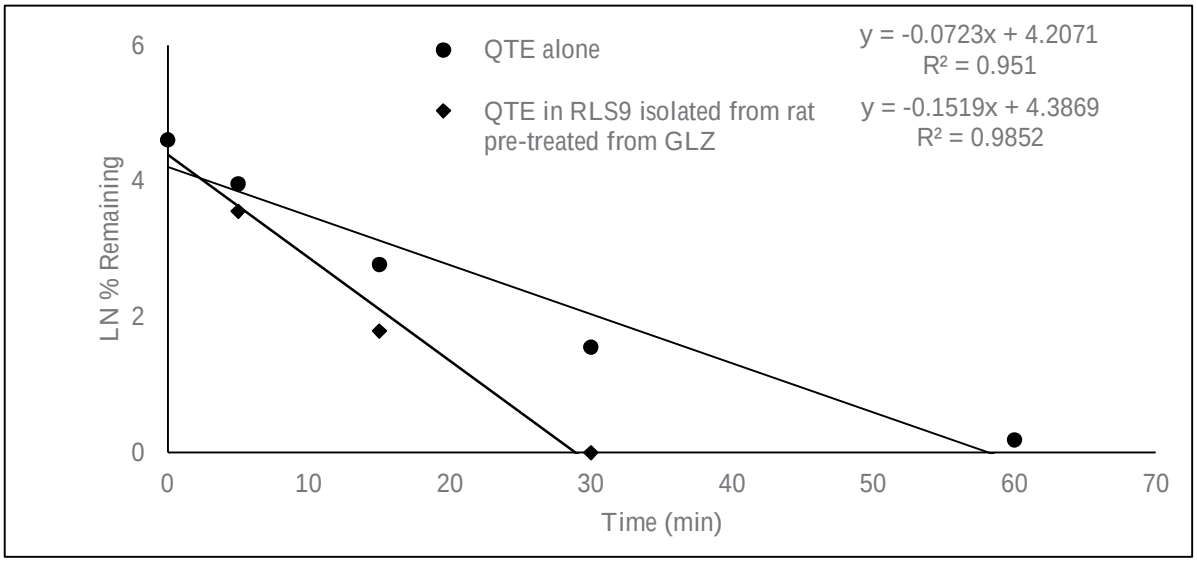



**Figure 6.10.** Semi-log plot of QTE remaining *Vs.* time (minutes) incubated in RLS9 isolated from control rats and rats pre-treated with GLZ.

Positive control worked as expected wherein complete inhibition was observed in the presence of itraconazole (100 ng/mL), suggesting that CYP3A4 was involved in the metabolism of QTE.

Seven-day *p.o.* administration of GLZ lead to significantly reduced systemic exposure of QTE. The pharmacokinetic studies established that GLZ could decrease the $t_{1/2}$ value and increase the clearance rate of QTE, and these outcomes were confirmed by using the RLS9 incubation experiments, as GLZ could accelerate the metabolism of QTE and escalate the intrinsic clearance rate. As QTE is extensively used in clinics, its potential interaction with licorice/GLZ pose a risk to patients with psychosis as the effective concentration of the latter in the plasma decreases when the standard dose of GLZ is co-administered. Accordingly, the patients who receive GLZ in the long-term should be cautious while on treatment using QTE.

### 6.6. Conclusion

Based on the observations from the rodent studies, no significant changes were observed on the pharmacokinetics of QTE after co-administration with HCA, and consequently no clinically relevant pharmacokinetic-based interaction is expected. When considering the frequent administration of the supplements containing *G. cambogia* and QTE, and considering the effects observed on pharmacokinetics of QTE, the therapeutic drug monitoring of QTE may be vital to confirm its efficacy. However, to establish a more reliable clinical evidence, it would be advantageous to design and conduct a clinical trial to precisely evaluate the safety during the co-administration of HCA and QTE

Our data suggest that QCN, a common ingredient in food and dietary supplements could significantly modify the pharmacokinetic profiles of QTE by changing the activity of CYP3A4 and P-gp. Significant increase in the levels of QTE, a narrow therapeutic index drug, in plasma and brain, when co-administered with QCN raises concern about herb-drug interactions potentially leading to toxicity. To date, this is the first report about the effects of QCN on the absorption and disposition of QTE.

In case of GLZ the *in vivo* study demonstrated that a 7-day pre-treatment with GLZ remarkably decreased the systemic exposure of QTE and increased the clearance of QTE that correlated well with *in vitro* results. Induction of DMEs and efflux transporter (CYP3A4/P-gp) might increase the metabolism and thereby clearance of QTE from the body. Thus, patients receiving QTE should be alerted when herbal dietary supplements containing GLZ are used for long-term. Further clinical research and therapeutic drug monitoring may be required to institute guidelines for concomitant use of GLZ and QTE .

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