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Influence of quercetin on amiodarone pharmacokinetics and biodistribution in rats

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Abstract. – OBJECTIVE: Amiodarone (AMD), a drug of choice to treat cardiac arrhythmias, has a narrow therapeutic index (NTI). It inhibits CYP3A4, CYP2C9, and CYP2D6 enzymes. Quercetin (QUE), a pharmacologically important bioflavonoid in vegetables and fruits, is important in treating cardiovascular comorbidities. QUE alters the bioavailability of drugs used concurrently by dual inhibition of P-glycoproteins (P-gp) and cytochrome (CYP) enzyme systems. The current study aimed to investigate the pre-treatment and co-administration effect of QUE on AMD pharmacokinetics in rats.

MATERIALS AND METHODS: Two separate animal trials (I and II) were planned to probe the effect of QUE on AMD pharmacokinetics by following previously cited studies. The pre-treatment group received oral doses of QUE for 14 days, and a single dose of AMD on the 15th day. Rats were administered single doses of QUE (20 mg/kg) and AMD (50 mg/kg) concurrently in a carboxymethylcellulose (CMC) in the co-administration study. Blood was collected at pre-determined time points. AMD was quantified by HPLC, and data was analyzed by PK solver software.

RESULTS: In the pre-treated group, peak plasma concentration (C_{max}) and area under the curve ($AUC_{0-\infty}$) of AMD were increased by 45.52% and 13.70%, respectively, while time to achieve maximum concentration (t_{max}), half-life ($t_{1/2}$) and clearance (CL) were declined by 35.72%, 16.75%, and 11.0% respectively compared to the control. In the co-administered group, compared to controls, C_{max} and $AUC_{0-\infty}$ were elevated to 12.90% and 7.80%, respectively, while t_{max}, $t_{1/2}$, and CL declined by 16.70%, 2.35%, and 13.40%. Further, AMD was increased in lung tissue of both treated groups, relative to the respective controls.

CONCLUSIONS: A notable pharmacokinetic drug interaction between QUE and AMD was observed in rats and warrants possible drug interaction study in humans, suggesting AMD dose adjustment specifically in patients with arrhythmia having a pre-treatment history and simultaneous administration of QUE-containing products.

Key Words:
Quercetin, Amiodarone, Bioavailability, Pharmacokinetic herb-drug interactions.

Abbreviations

AMD, amiodarone; CYP, cytochrome; CMC, carboxymethylcellulose; H&E, hematoxylin-eosin; HDIs, herb-drug interactions; MDR1, multidrug resistance protein 1; MRP-2, multidrug resistance protein 2; NTI, narrow therapeutic index; P-gp, P-glycoprotein; QUE, Quercetin.

Introduction

Patients may prefer the simultaneous use of herbal therapies with allopathic drugs to relieve the same ailments or other comorbidities. In addition, the concomitant use of herbal and allopathic drugs may lead to clinically relevant herbal drug interactions (HDIs) because the former constitutes several highly bioactive constituents[1]. HDIs are frequently unrecognized by medical experts, herbalists, and patients[2]. The use of alternative therapies for the treatment of heart disease is rising. Heart patients commonly use herbal and

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prescribed allopathic cardiovascular medications, posing a risk of HDI. One of the most common flavonoids in the diet is QUE. It is mainly found as glycoside derivatives widely distributed in plant-derived foods[3] and can be ingested by eating various vegetables and fruits. Onion, apple, and red wine are rich sources of QUE[4]. It is non-toxic and has the following properties: anti-cancer[5], neuroprotective[5], anti-oxidant[6], anti-viral[7], anti-ulcer[8], anti-allergic[9], anti-inflammatory[10], and anti-diabetic[3,11].

QUE inhibits several cytochrome P450 (CYP) isoenzymes, i.e., CYP3A4, CYP2C8 CYP2C9 and CYP1A2[12,13]. It also inhibits the P-glycoprotein (P-gp) efflux transporter, multidrug-resistant protein 1 (MRP1), and breast cancer-resistant protein (BCRP)[14,15]. P-gp is widely expressed and distributed in the intestinal epithelium, where it impels drugs back into the intestinal lumen. In the liver, P-gp effluxes xenobiotics in the liver cells, which propels them into the bile duct. QUE thus has the potential of being involved in HDIs when used concomitantly with drugs. The prominent effect on the CYP enzyme system could alter the bioavailability of co-administered drugs[16,17], especially those with NTI drugs, leading to life-threatening side effects[18].

Amiodarone (AMD) (Figure 1) is one of the most widely prescribed drugs for treating atrial fibrillation and ventricular arrhythmias[19]. However, its pharmacokinetics is mostly uncommon and challenging from a pharmacological standpoint[20,21]. AMD is a highly lipophilic molecule with varied oral bioavailability (20-80%). Furthermore, AMD has a restricted therapeutic window (0.5-2.0 mg/mL) linked to significant clinical drug interactions[20,22-26]. AMD affects the eyes, liver, lungs, and thyroid. AMD-induced toxicity is ranked in the liver up to 50% > thyroid up to 22% > pulmonary up to 7%. The long $t_{1/2}$ of AMD (20.73±14.05 h) and high incidence of organ toxicity suggest careful monitoring of the patients on AMD[27,28]. AMD is the potent inhibitor of CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP2C19 CYP2D6 and CYP3A4[29,30]. It is the substrate of P-gp and metabolized by CYP2C8, CYP2C19, and CYP3A4[30-34]. Mono-N-desethylamiodarone (MDEA) is a prime metabolite of AMD and is produced through the most common CYP isoenzyme-let metabolic pathway[30,35,36]. MDEA results in a large Vd and variable tissue accumulation[21], making it a clinically significant metabolite[30]. It is also a CYP inhibitor[32]. Considering all of the above factors and the possibility of simultaneous use of QUE and QUE-containing products with AMD, the present study was designed to investigate the effect of QUE on the pharmacokinetics of AMD in rats.

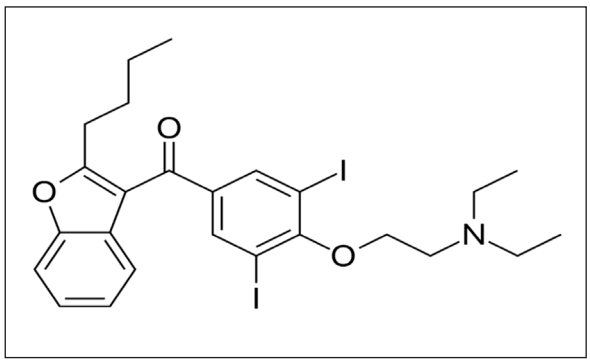

Figure 1. Structure of amiodarone.

Materials and Methods

Materials

AMD (99.5%, Fengchen Group Co. Ltd, Qingdao, China) and tamoxifen (98.10%, Shaanxi Kang New Pharmaceutical Co., Ltd, Xi'an China) were gifted by Schazoo Zaka (Pvt) Ltd., Lahore Pakistan. CMC (Aashi Chem, Surat India) was provided by PharmaWise (Pvt) Ltd., (Lahore Pakistan). Formic acid, Potassium phosphate monobasic, and acetonitrile (Sigma Aldrich, St. Louis, MO, USA), quercetin (Fluka, Buchs, Switzerland), and Methanol (Merck, Darmstadt, Germany), were procured from the local market in Lahore Pakistan.

Animals

The experimental work was carried out using Wistar albino rats of either sex weighing between 189-242 g. Under the standard environmental conditions, rats were acclimatized for one week before the study. The animal trial was performed after formal approval from the Animal Ethical Committee, Punjab University College of Pharmacy, University of Punjab (AEC/PUCP/1077), dated 03-05-2018, according to universally accepted protocols.

Experimental Design

Two separate animal trials (I and II) were planned to probe the effect of QUE on AMD pharmacokinetics by following previously cited studies[1,37-39].

Trial I: Pre-treatment with QUE for 14 Days and Then AMD on 15th Day

In Trial I, the rats were divided into control and pre-treated groups (n = 6, each). The control group was given 2 ml of vehicle (0.5% CMC) orally for 14 consecutive days. On the 15th day, animals were dosed 50 mg/kg, p.o AMD in the vehicle. Similarly, pre-treated animals received QUE (20 mg/kg, p.o) in CMC (0.5%) for 14 consecutive days. The administration of the substances to the animals was done through oral gavage. AMD dose was selected based on the reported HDI studies between AMD and Fucus vesiculosus, Citrus aurantium, Carica papaya and Paullinia cupana[1,37-39]. Likewise, QUE's dose was chosen based on a HDIs study reported between QUE and saxagliptin [40]. In addition, animals were weighed before the commencement of the study, i.e., on day 1 and the last day of the study, i.e., on 15th day to probe the effect of QUE on the bodyweight of animals.

Trial II: Simultaneous Administration of QUE and AMD

In Trial II, the control group (n = 6) received an AMD single dose (50 mg/kg, p.o) in the CMC (0.5%). At the same time, the co-administered group was simultaneously administered with QUE single dose (20 mg/kg, p.o) and AMD (50 mg/kg, p.o) in CMC (0.5%).

Blood and Tissue Sampling

Blood samples (around 0.5 mL) were collected at nominal time-points 0.25, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, and 12.00 h post-dosing from the rats' orbital sinus under the phenobarbital sodium anesthesia into the heparinized tubes. The collected samples were centrifuged at 4,000 rpm for 10 min at 4°C. The separated plasma was stored at –20°C for chromatographic analysis. After 12 h post-dosing in both trials, the rats were sacrificed under anesthesia to collect the vital organs (heart, liver, lungs, and kidneys). We weighed the organs before homogenization in 3 ml of purified water per 3 g of tissue to get the tissue homogenates for analysis[39].

Extraction Procedure for Biological Samples

For extraction of AMD from plasma, 150 µL of plasma was mixed with 0.1 M Na_3PO_4 buffer and added to 20 µL of tamoxifen as internal standard having a concentration of 50 µg/mL in water-methanol 60:40 v/v. Then 500 µL of n-hexane was added, mixed for 30 sec, and centrifuged for 2 min at 17,000 rpm at 4°C. The upper organic layer was collected in glass vials, and the same procedure was repeated thrice. The solvent was evaporated, the residue was reconstituted with 200 µL of methanol, and injected into the liquid chromatograph.

For the extraction of AMD from tissues, 400 µL of tissue homogenates were mixed with 20 µL of internal standard and 400 µL of acetonitrile, followed by vortex mixing. The whole mixture was centrifuged at 4°C for 10 min at 17,000 rpm. The supernatant was separated, and 1 mL of n-hexane was added and again centrifuged as previously described. The rest of the procedure was the same as described for the extraction of plasma samples.

Determination of AMD in Plasma Samples and Tissues Homogenates

A reported liquid chromatographic method was employed for the determination of the AMD in the plasma and tissue homogenates[41] by using LC-20-A HPLC system equipped with SPD 20-A detector (Shimadzu) (Shimadzu Scientific Instruments, INC. 7102 Riverwood Drive, Columbia, Maryland, USA). The High Performance Liquid Chromatography (HPLC) conditions consisted of 20 µl injection volume, C18 column (LiChroCART 55 x 4 mm, 3 µm Puroshper STAR RP-18 end capped) having a flow rate of 1.2 mL/min at detector wavelength of 254 nm. The mobile phase comprised 50 mM formic acid buffer: methanol: acetonitrile 45:5:50 v/v/v, filtered and degassed using 0.45 µm membrane filters (Millipore, Bedford, USA). The pH of the buffer solution was adjusted to 3.10 with 0.1% formic acid solution. Before quantitative AMD analysis in biological samples, the HPLC method was validated for precision, accuracy, linearity, range, specificity, robustness, solution stability, stress, limit of detection and limit of quantification[42].

Pharmacokinetic Assessment

The plasma-concentration-time data were used to calculate the following pharmacokinetic parameters with non-compartmental analysis employing PK-Solver tool (PK Solver Tongjiaxiang, Nanjing, China): peak plasma concentration (C_{max}), time to attain C_{max} (t_{max}), area under the curve from the initial time (time zero) to the last time interval (AUC_{0-t}), total AUC ($AUC_{0-\infty}$), mean residence time (MRT), the volume of distribution (Vd), rate of elimination (Ke), half-life ($t_{1/2}$), and clearance (CL).

Table I. The recovery analysis of plasma and tissue homogenates for amiodarone and The inter-day and intra-day precision and accuracy for amiodarone in plasma.

Concentration (µg/ml)	Recovery (n = 3) (pasma)		Recovery (n=3) (tissues)		Intra-day (n = 6)			
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Accuracy (% of true value)	Precision RSD (%)	Accuracy (% of true value)	Precision RSD (%)
50	86.80	1.94	86.80	1.94	94.45	4.53	92.86	2.26
100	96.52	1.10	96.52	1.10	96.59	0.80	94.72	1.70
150	100.69	1.87	100.69	1.87	98.52	1.74	97.90	1.87

RSD = relative standard deviation

Statistical Analysis

Data were presented as the mean ± SEM. Pre-treated and co-administered experimental groups were compared with respective controls using the Mann-Whitney U-test. Statistical parameters were calculated through Graph Pad Prism® Version 8.0.1.244 for Windows (La Jolla, CA, USA). A $p < 0.05$ was considered significant.

Results

Validation of HPLC Bioanalytical Method

The detail of validation parameters of the bioanalytical method for AMD analysis in plasma and tissue homogenates is shown in Tables I, II, and III, while the representative chromatograms for blank, lower limit of quantification (LLOQ) and study sample are shown in Figure 2.

Effect of QUE on AMD Plasma Level Data Time

In pre-treatment group, plasma concentration of AMD remained higher till 8 h (Figure 2A) but at 0.5 h, 1.0 h and 2.0 h, AMD was significantly higher ($p < 0.05$) than that in the control group, i.e., 2.20 µg/mL *vs.* 1.40 µg/mL, 2.88 µg/mL *vs.* 1.76 µg/mL, and 3.48 µg/mL *vs.* 2.26 µg/mL, respectively at the above time intervals. In the co-administration group, the AMD concentrations were higher till 8 h and were lesser at the last time point than that of the control group (Figure 2B). The magnitude of concentration increase was greater in the pre-treated group than in the

Table II. The solution stability and robustness for amiodarone in plasma.

Solution stability (n = 3)				Robustness (n = 3)	
Temperature (°C)	Time (h)	Assay (%)	RSD (%)	Parameters	RSD (%)
20	1	96.59	1.23	Wavelength ± 2 nm	0.48
20	12	94.81	1.41	Flow rate ± 15%	0.35
20	24	93.21	1.60	Mobile phase ratio ± 3%	0.32
–20	1	94.58	1.47	pH ± 0.2	1.22
–20	12	93.01	1.70		
–20	24	91.29	2.03		

RSD = relative standard deviation.

Table III. The values of R^2, Linearity, LOD and LLOQ for amiodarone in plasma.

Specificity	R^2	Linear range (µg/ml)	LOD (µg/ml)	LLOQ (µg/ml)
Retention time of standard and sample matches	0.998	0.20 - 20.00	0.05	0.20

R^2 = coefficient of determination, LOD = lower limit of detection, LLOQ = lower limit of quantification.

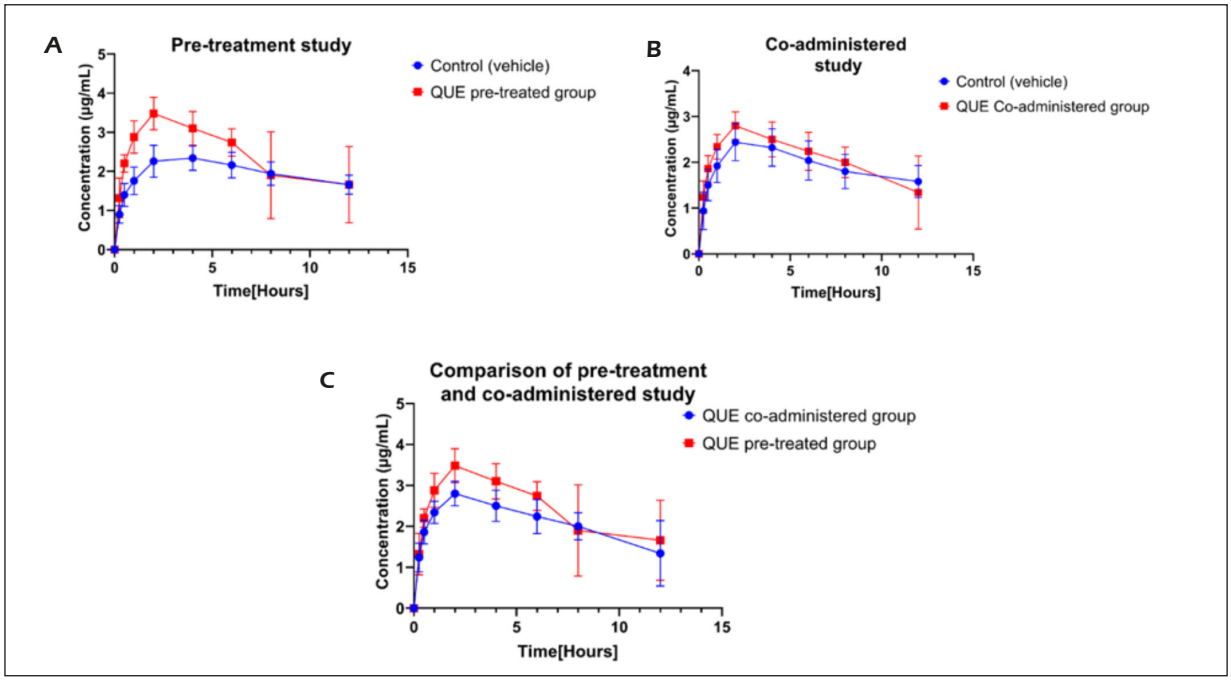

Figure 2. Plasma level time curve of amiodarone in (**A**) pretreatment, (**B**) co-administration groups, and (**C**) comparative plasma drug levels in pretreatment and co-administration. (QUE = quercetin).

co-administration group, as shown in Figure 2C.

An increase in AMD concentration in both treatment groups was due to increased absorption of AMD across the intestinal membrane *via* passive diffusion, possibly on account of the competitive inhibition of intestinal CYP3A4, CYP2C9, and CYP1A2 and P-gp by QUE[43], leading to a lesser AMD prehepatic metabolism. Furthermore, QUE could increase the rate at which AMD passes into the intestine by increasing gastrointestinal motility, as reported by Kim et al[44]. As a result, more drug was absorbed from the duodenum, jejunum, and small intestine in both the experimental groups compared to the control (Table IV).

Effect of QUE on AMD Pharmacokinetic Parameters

In the QUE pretreatment group, a rise of 45.52% in C_{max} and 13.70% in $AUC_{0-\infty}$, while

Table IV. Comparative pharmacokinetic parameters (mean ± SEM, n = 6) of amiodarone from control, quercetin pre-treated and co-administered groups.

Parameter	Pre-treated group			Co-administered Group		
	Control	QUE	Trend (%)	Control	QUE	Trend (%)
t_{max} (h)	2.8 ± 0.50	1.80 ± 0.40	↓ 35.72	2.4 ± 0.40	2.0 ± 0.10	↓ 16.7
C_{max} (µg/mL)	2.46 ± 0.30	3.58 ± 0.40	↑ 45.52	2.48 ± 0.20	2.80 ± 0.30	↑ 12.9
AUC_{0-t} (µg.h/mL)	23.60 ± 1.4	28.73 ± 3.42	↑ 21.73	23.20 ± 1.90	24.30 ± 1.60	↑ 4.70
$AUC_{0-\infty}$ (µg.h/mL)	61.80 ± 4.20	70.26 ± 6.49	↑ 13.70	60.60 ± 7.90	65.32 ± 5.63	↑ 7.80
MRT (h)	23.85 ± 0.70	19.73 ± 1.3	↓ 17.28	23.94 ± 2.20	23.25 ± 1.30	↑ 2.90
Vd (L)	18.90 ± 1.12	13.82 ± 0.70	↓ 26.88	20.05 ± 2.35	17.84 ± 1.30	↓ 11.03
Ke (1/h)	0.042 ± 0.1	0.05 ± 0.01	↑ 19.04	0.04 ± 0.1	0.04 ± 0.01	----
$t_{½}$ (h)	15.94 ± 1.30	13.27 ± 0.90	↓ 16.75	16.20 ± 1.60	15.82 ± 0.90	↓ 2.35
Cl (L/h)	0.82 ± 0.12	0.73 ± 0.21	↓ 11.00	0.90 ± 0.12	0.78 ± 0.10	↓ 13.40

QUE = Quercetin, t_{max} = time for maximum concentration, C_{max} = Maximum drug concentration, AUC = area under the curve, MRT = Mean residence time, VD = volume of distribution, Ke = Elimination rate constant, $t_{1/2}$ = Half life and Cl = Clearance.

a 35.72% decline in t_{max} ($p > 0.05$) was noted. The disposition parameters of AMD, like MRT, Vd, $t_{1/2,}$ and CL, were reduced, respectively, by 17.28%, 26.90%, 16.75%, and 11.00% in the presence of QUE relative to the control group. The pre-treated group showed a notable difference ($p > 0.05$) for C_{max}, t_{max}, and Vd compared to the control. A similar trend in the QUE co-administration group (Trial II) was noted, with a 12.9% increase in C_{max} and 7.80% in $AUC_{0-\infty}$, while a 16.70% decrease in t_{max} was observed. The Vd was reduced by 11.03%, $t_{1/2}$ by 2.35%, and CL by 13.40%, respectively. However, in the QUE co-administered experimentation, the difference in absorption and disposition parameters compared to the control group was not significant ($p > 0.05$).

As reflected by the plasma concentration data and increased C_{max}, $AUC_{0-\infty}$ and decreased t_{max}, the simultaneous administration of QUE with AMD increased the extent and rate of systemic exposure of AMD compared to the control due to the increased intestinal absorption[44]. The higher exposure of AMD in the QUE pre-treated group in comparison to the co-administered group could be attributed to the dual inhibition of the intestinal MDR1, MRP-2, P-gp, and prehepatic CYP450 system in the presence of QUE, which seemed to be time-dependent, as reported[45]. This prehepatic effect was more prominent in the pre-treated group as it showed notable differences ($p > 0.05$) for C_{max}, t_{max}, and Vd compared to the control. The dual substrate for transporters and CYPs has a higher interaction potential with the same category of phytomedicines[46].

Effect of QUE on Body Weight

The weight of rats in both groups was significantly increased on 15th day as compared to that on the day 1st (Figure 3), which might be due to the nutritional effects of the vehicle, CMC.

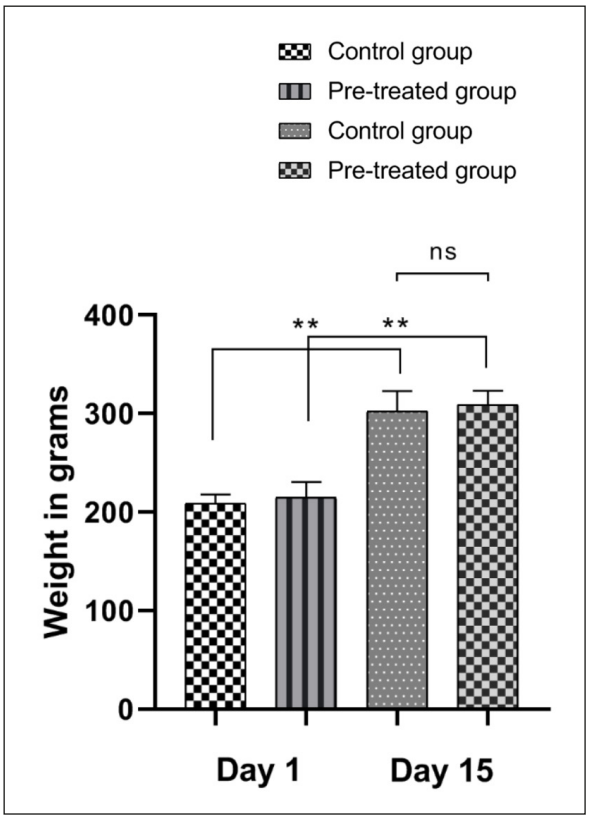

Figure 3. Effect of QUE pre-treatment on rat body weights. **Indicates $p < 0.01$.

Effect of QUE on Organ Distribution of AMD

Exploring the effect of QUE on the biodistribution of AMD has toxicological significance in the tissues or organs through customized toxicity trials. The mean concentration of AMD in lungs, kidneys, liver and heart of animals of Trial I was notably higher ($p > 0.05$) compared to control at 12 h post-dosing (Table V). A statistically significant difference in the mean tissue concentration of AMD was noted in lungs (16.70 ± 2.69 µg/ml,

Table V. Tissue AMD concentration (mean ± S.E.M, n=6) in pre-treatment and co-administered groups at 24 h after QUE dosing.

Experimental groups	Concentration (µg/ml) of AMD			
	Lungs	Kidney	Liver	Heart
Control group I	9.26 ± 2.97	2.8 ± 1.6	1.86 ± 1.60	1.38 ± 1.40
Experimental (co-administered)	15.20 ± 3.01	5.78 ± 1.98	3.36 ± 1.30	1.76 ± 0.60
Control group-II	10.74 ± 2.95	4.18 ± 1.82	3.84 ± 1.86	1.0 ± 1.40
Experimental (pre-treatment)	16.70 ± 1.20	6.70 ± 1.76	6.30 ± 1.30	1.80 ± 1.49

AMD: amiodarone.

$p > 0.05$) followed by kidney (6.70 ± 3.94 µg/ml, $p > 0.05$), liver (6.30 ± 2.9 µg/ml, $p > 0.05$) and heart (1.80 ± 1.11 µg/ml, $p > 0.05$) in the pre-treatment group compared with control.

The mean concentration of AMD in the lungs, kidneys, liver, and heart of animals of Trial II was also found to be notably higher ($p > 0.05$) compared to the control at 12 h post-dosing (Table III). An insignificant difference in the mean tissue concentration of AMD was noted in lungs (15.20 ± 3.01, $p > 0.05$) followed by kidney (5.78 ± 1.98, $p > 0.05$), liver (3.36 ± 1.80, $p > 0.05$) and heart (1.76 ± 1.25, $p > 0.05$) in a co-administered group compared to the control. The tissue distribution of AMD was noted in the following order: lungs > kidney > liver > heart. The QUE pre-treated group showed higher AMD concentration in organs than the co-administered group (Figure 4A and Figure 4B). Lungs showed higher drug concentration in both experimental groups than the control and other organs. No literature reports are available regarding the effect of QUE on AMD tissue concentrations to date.

Discussion

The possibility of HDIs exists on the simultaneous use of QUE with other medications[12,16,17]. The simultaneous usage of QUE and AMD may easily be found due to the abundance of QUE in dietary supplements. When it comes to NTI drugs, like AMD, the area of HDIs sparks further significant concern. Before conducting clinical trials, evaluating the interactions between phytomedicines and synthetic pharmacotherapeutic drugs in animals is required, while most of the HDI has been assessed *in-vitro*[47], with drugs at higher concentrations than those used in the clinical practice. In line with the above, the current in-vivo study was designed to probe the effect of QUE on the pharmacokinetics of AMD in rats, in pre-treatment and co-administration groups. As far as we know, the current research work reports the HDIs between QUE and AMD for the first time in rats. Though the data from the animal studies cannot be directly applicable to humans, yet the rat appears to be a viable model for studying HDIs[48,49].

Drug interactions mainly occur because of inhibition or induction of enzyme systems or masking of transporters. Induction of CYPs, being time-dependent, is a slower process and may take 7-10 days; thus, the effect of QUE on the pharmacokinetics of AMD was studied by pre-treating the animals in Trial I with QUE (20 mg/kg/day, p.o.) for 14 consecutive days before administrating AMD single dose (50 mg/kg/day, p.o.) on the 15th day. It also mimics a condition where the patients are already on QUE and also starts the allopathic treatment[2]. The transporter and metabolism induction occurs within 24-48 hours post-dosing, leading to the co-administration study in this investigation.

QUE is a globally recognized safe complementary or alternative medicine used for different cardiovascular comorbidities[50]. However, it may increase the systemic exposure of medications on concurrent use. QUE inhibits multi-CYP enzymes and transporters in a concentration-dependent manner. The inhibition of CYP3A4 has clinical importance, as it metabolizes around 60% of drugs, which leads to critical drug interactions[51] and accumulation of parent drugs that may increase the risk of side effects and toxicity. CYP2C8 is highly expressed in the human liver and is known to metabolize more than 100 drugs. Similarly, inhibition of CYP2C9 elevates the con-

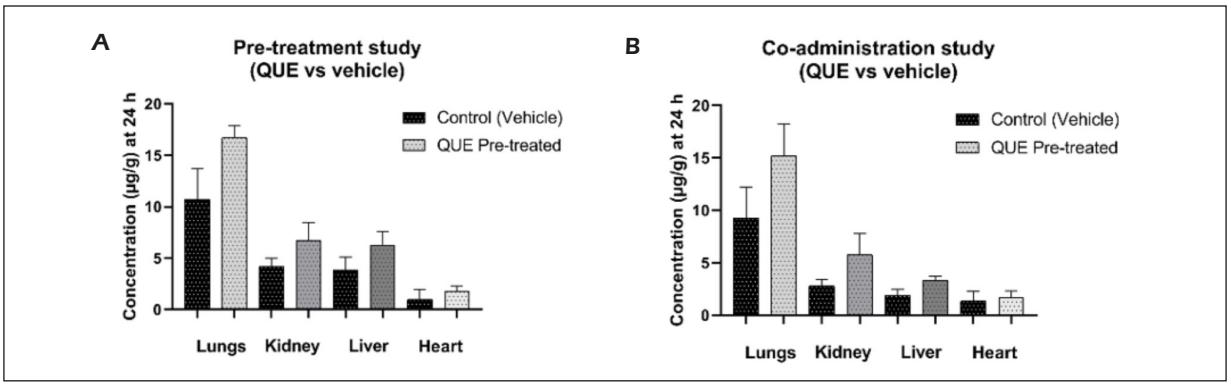

Figure 4. Biodistribution of AMD in different rat organs of pre-treated and co-administered groups. QUE = Quercitin.

centration of certain drugs, leading to drug toxicity[52]. Previous research[1,37-39] unfolds various HDIs between AMD and Fucus vesiculosus Citrus aurantium, Carica papaya and Paullinia cupana extract in rats. Grapefruit juice has already been reported to inhibit AMD metabolism, leading to its enhanced concentration, though the relevance of this interaction on the long-term efficacy and toxicity has yet to be established[53]. The systemic exposure of AMD and MDEA is also reduced substantially by their simultaneous use with orlistat, an anti-obesity drug[54]. While the MDEA has been reported to be increased, showing elevated metabolism of AMD in rats exposed to β-naphthoflavone through the CYP induction[32].

The findings of the present studies were in line with the previous studies, where the concurrent use of AMD with other phytomedicines resulted in a prominently increased drug systemic exposure and decreased $t_{1/2}$[38,39]. However, a change lesser than 20%, a general criterion for equivalence, might not be clinically significant.

The reduction in disposition parameters, including Vd, $t_{1/2}$, and CL, indicated the enhanced metabolism of AMD. The QUE pre-treated group showed a briefer $t_{1/2}$ with a greater magnitude than that observed in the co-administration group. As a general principle, $t_{1/2}$ increases with enhanced drug exposure. Contrarily, in the pre-treated group compared to the co-administered group, Vd, $t_{1/2}$, and CL altered by 22.54%, 16%, and 6.50%, respectively, which was lower than 20%, stipulated for a relevant clinical difference. A lower value of Vd in the QUE pre-treated group relative to the co-administered group indicated more drug in blood than the tissues. Similarly, lesser CL corresponded to the lower value of $t_{1/2}$. Furthermore, the CL determines the magnitude of drug distribution and elimination, which may likely become lower when AUC is higher for a given drug dose[37]. On the other hand, CL decreased in the pre-treated and co-administered groups, being with a higher magnitude in the co-administered group than the pretreatment group in the presence of QUE. Decreased values of disposition parameters might result from the induction of hepatic CYP with greater magnitude compared to the QUE co-administered group. Thus, a rapid and increased AMD exposure caused a higher plasma concentration followed by an increased drug metabolism in the QUE-treated groups, in line with the literature[38,39]. Masking of intestinal CYP and transporters in prehepatic metabolism and induction in hepatic CYP might be explainable, possibly due to independent regulation and lack of structural similarity between the hepatic and intestinal CYP enzymes. Thus, CYP3A4 inhibitors may increase plasma concentration by reducing pre-systemic metabolism more than the systemic metabolism of certain drugs. Literature supports the above, whereby cyclosporine, a potent CYP3A4 inhibitor, increases statin exposure (AUC) without any significant impact on $t_{1/2}$. Grapefruit juice has been shown to increase the systemic exposure of felodipine with a decreased $t_{1/2}$ of the drug compared to water, as control[55]. Another study[56] suggests that grapefruit juice increases the exposure of felodipine and nifedipine without significantly affecting the $t_{1/2}$. The same has also been reported for AMD in case of interaction with other phytomedicines[38,39]. As stated above, MDEA is also a CYP inhibitor[32], yet its role in the elevation of absorption parameters and decreased disposition could not be explainable in the present study.

The pre-treatment with the repeated and single co-administration of QUE and AMD on one occasion influenced the AMD pharmacokinetics in rats. Pre-treatment with QUE remarkably increased the systemic exposure of the AMD relative to that of AMD in the co-administration, probably due to the masking of the P-gp, which inhibited the AMD biotransformation but elevated accumulation in the lung tissue. The current study was performed in rats, and the same impact of QUE on AMD might not be predicted in humans, yet the findings provided evidence to warrant a drug interactions study in humans. The higher systemic exposure of AMD might have clinical implications, which after confirmation, might be expected to alter efficacy, toxicity, thyroid functions, cause hepatic injury, *cornea verticillata,* and pulmonary ailments on long-term use because of the elevated AMD systemic concentrations[57-59]. Thus, studying QUE-AMD interaction in humans could help confirm the safe and effective AMD treatment.

Conclusions

Quercetin affected the bioavailability of amidone in rats in pre- and co-administered groups, being with higher magnitude in the pre-treatment group. In both groups, amidone showed significantly higher peak plasma concentration and area under the curve compared to control. Decrease

in the time to peak blood drug concentration indicated a faster drug absorption in presence of quercetin. The enhanced drug exposure was further supported with a briefer half-life and clearance, compared to the control. Further, drug concentration was increased in lung tissue, relative to the respective controls. The increased amidone exposure in the presence of quercetin in rats might have the clinical implication, warranting confirmation. In a further study, it should be noted that whether the above increase in bioavailability might (a) be beneficial in improving drug concentration and thereby, antiarrhythmic effect, (b) be advantageous to improve the drug resistance (c) require a reduced drug dose, (c) lead to drug accumulation in the lungs and overall toxicity with long term usage of quercetin or (d), warrant drug dose adjustment in patients with an irregular heartbeat to avoid toxicity, when they are pretreated with quercetin or its products.

Conflict of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All the authors declare no conflict of interest.

Ethics Approval
All the animal studies were carried out according to internationally accepted protocols, and it was approved by the Institutional Animal Ethical Committee College of Pharmacy, University of Punjab (AEC/PUCP/1077) dated 03-05-2018.

Informed Consent
Not applicable.

Authors' Contribution
Conceptualization, N.I. Bukhari; methodology, E. Ahmad; software, M. Jahangir; validation, J. Khan; formal analysis, A. Sarwar.; investigation, T. Aziz; resources, A. F. Alasmari; data curation, M. Alharbi and A. Alsahammari; writing-original draft preparation, E. Ahmad.; writing-review and editing, G. Nabi; visualization, T. Aziz; supervision, T. Aziz; project administration, N.I. Bukhari; funding acquisition, T. Aziz.

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