Original Article

Enhancing the Bioavailability of Quercetin by Concomitant Administration with Enzyme Inhibitor

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Abstract

Background: Quercetin is a food supplement with multiple biological activities including antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic, antiatherosclerotic and other activities, it is available in wide range of dietary supplement and daily food intake, after oral ingestion, quercetin is metabolized by intestinal enzymes and absorbed by the duodenum, then be transported to the liver where is transformed into its glucuronidated, methylated and sulfo-substituted metabolites.

Aim of Study: Boosting the bioavailability of Quercetin by enzyme inhibitor effect of ketoconazole

Method: 10 apparently healthy adult, male (5) and female (5) were grouped A and B, with a 1-week wash-period, Group A received Quercetin 1000 mg, group B received Quercetin 1000 mg and Ketoconazole 400 mg, and 15 blood samples are collected on the following timing 0, 0.5 - 12.5 by 1 hour interval and other by hour 24 for HPLC Assay.

Results: Concurrent ketoconazole administration with Quercetin significantly improves the mean plasma concentration under the curve from zero time to infinity (AUC0–∞) by 176% (403.41 ± 62.28 vs. 228.51 ± 28.67 µg.h/mL; P <0.0001) and prolonged time to complete elimination (44.66 ± 9.17 vs. 12.93 ± 1.4 hr.; P <0.0000) compared with Quercetin alone.

Conclusion: Bioavailability of Quercetin will be increased by its administration together with ketoconazole, due to enzyme inhibiting effect of ketoconazole which delay elimination and increase the AUC of Quercetin.

Keywords: Quercetin, Ketoconazole, bioavailability, enzyme inhibitor.

INTRODUCTION

Quercetin (Que) is a pentahydroxyflavone (C15H10O7). (1, 2) It is polyphenolic flavonoid compound (3, 4) used as a food supplement and has numerous biological activities; (5) it is taken as a single ingredient or in combination with other vitamin supplements. (6, 7) Que is a needle crystal of brilliant citron yellow it is insoluble in frigid water, poorly soluble in wormed water, while it is quite soluble in the lipids and alcohol. (5) The term "quercetin" has been used since 1857; (1) (8) it is originated from quercetum or quercus which means oak wood forest. (9) It is an aglycone, (lacking an attached sugar molecule). (10) Because the presence of hydrophilic sugar moiety which improves the solubility of a glycosyl group, the Que glycoside is soluble in water. (11) Que and its derivatives are of the most powerful antioxidants found in plants and contains several OH groups in its structure, which may further contribute to photodegradation. (8, 12) It is one of more than 4000 plant phenolics that are naturally available, (13) in many vegetables, fruits and drinks ingested from the daily diet, (14) including onions, broccoli, apples, berries, (15) and citrus fruits, (9) it is ubiquitously present in wine and tea. (16, 17)

Que is well known for its vasodilator, antihypertensive effects, antiatherosclerotic, antiobesity, antihypercholesterolemic, and antidiabetic activities. (18-20) Que's mechanisms of action are pleiotropic, involves the decrease of glucose absorption from the intestine, insulin secretory and insulin-sensitizing effects, and the enhancement of glucose uptake in the muscles. (21) Epidemiological studies indicate an inverse association between dietary intake of Que and cancer; (22) Que is empirically used for the treatment of asthma, hay fever, eczema, hives, and gout as examples of allergic disorders (14) Moreover, flavonoids

are attributed with clinically relevant functions, such as the stabilization of mast cells, and are known for their antiinfertility, antitumor, and antihepatotoxic activity. (13) Their biological antioxidant activities include preventing the oxidation of low density lipoproteins in vitro and anticarcinogenic activities. Que is a one-of-a-kind bioflavonoid that has been intensively researched in recent years. (9)

Que is usually present in glucuronidated, sulfated, or methylated forms in human plasma. (23) It is found in plants in the form of glycosides, which are transformed into aglycones in the colon by -glycosidases before being absorbed by enterocytes. (24) Early studies on the pharmacokinetics of Que in humans revealed that it has relatively low oral bioavailability (2% after a single oral dosage). In healthy persons ingesting 100 mg of Que glucoside, the estimated absorption varies from 3% to 17%.(25) Its half-life (t1/2) was found to be between 0.8 and 2.4 hrs. (26, 27) The Glucosides of Que are mostly absorbed by the upper segment of the small intestine, where they are efficiently hydrolyzed to aglycone form by β-glucosidases, much of which is subsequently absorbed and finally excreted in urine by the kidneys. (5) Gastric acid does not affect the stability of Que or its derivatives. (25) The continuous consumption of Que-rich food considerably raised the concentration of Que in the blood plasma, and considerably raised Que concentration in plasma, which was found to be strongly related to its dietary content. (25) The concentration of Que in urine increased with increasing dosage and duration following the consumption of fruit juice in humans: benzoic acid derivatives are prevalent metabolites of Que, and within a few hours, these metabolites are delivered into the blood and then excreted renally. (28) Dietary Que supplementation is available, with a recommended daily dosage of 200 - 1200 mg. (23)

A major problem with Que is its low bioavailability because it has low lipo-solubility, (29) is poorly absorbed, and is rapidly metabolized (25) this restricts its medicinal use, despite the fact that it possesses a wide range of pharmacological activities. (8) another factor attributed to its low bioavailability might be its digestive tract's poor dispersion capacity. (28) It is quite unlikely that its maximum blood concentration (Cmax) exceeds 302 µg/mL (10 µM), regardless of whether a much greater oral dose of Que is given. (28)

Many methods have been used to boost the bioavailability of Que, including enhance absorption for example: prepared solid dispersions, self emulsifying drug delivery systems in addition to phytosomes, (29) decreasing metabolism and/or delaying elimination. (30) The use of drug-drug interaction with enzyme inhibitor is one of the approaches to enhance bioavailability by avoiding decomposition or metabolism in the gastrointestinal tract and delivering them directly to the blood and plasma. (31)

Ketoconazole (KETO) is a synthetic, (32) antifungal agent (33) in both animals and humans, it was found to be a P450 inhibitor. As a result, pharmacological interactions with other chemicals metabolized by the P450 monooxygenase system are possible with this imidazole molecule.(34, 35) After oral administration Que is rapidly absorbed and undergoes extensive metabolism in the liver, which may result in the formation of glucuronidated, sulfated, or methylated Que compounds, these metabolites have attenuated or unknown biological activates. (36) In general, the biological activities of Que, as well as other flavonoids, are directly correlated to the number of free hydroxyl groups; these OH groups are the main target of metabolism in the liver. (37) Que metabolites, do not exert pro-oxidant effects.(38) It had no influence on the contractile response. (39) Inhibiting these metabolism steps may preserve Que in its active form for a longer period of time; therefore using enzyme inhibitors such as KETO may result in such effects. (40)

Aims of study

Since Que is rapidly metabolized and eliminated, enhancing absorption and decreasing the elimination rate may improve its bioavailability. In this research the objective was to inspect the impact of the KETO enzyme inhibitor, on the bioavailability of Que and other pharmacokinetic parameters, such as AUC, Cmax, Tmax and the time to complete elimination, which has never been reported.

Study Approval

The protocols for this study were reviewed and approved by the Scientific and medical Ethics Committee of the Basra University College of pharmacy and all volunteers signed a document of informed consent that depend on Helsinki research ethics.

Study Design

Interventional double blind controlled study, involve administration of either Que 1000 mg, or Que 1000 mg and KETO 400 mg orally.

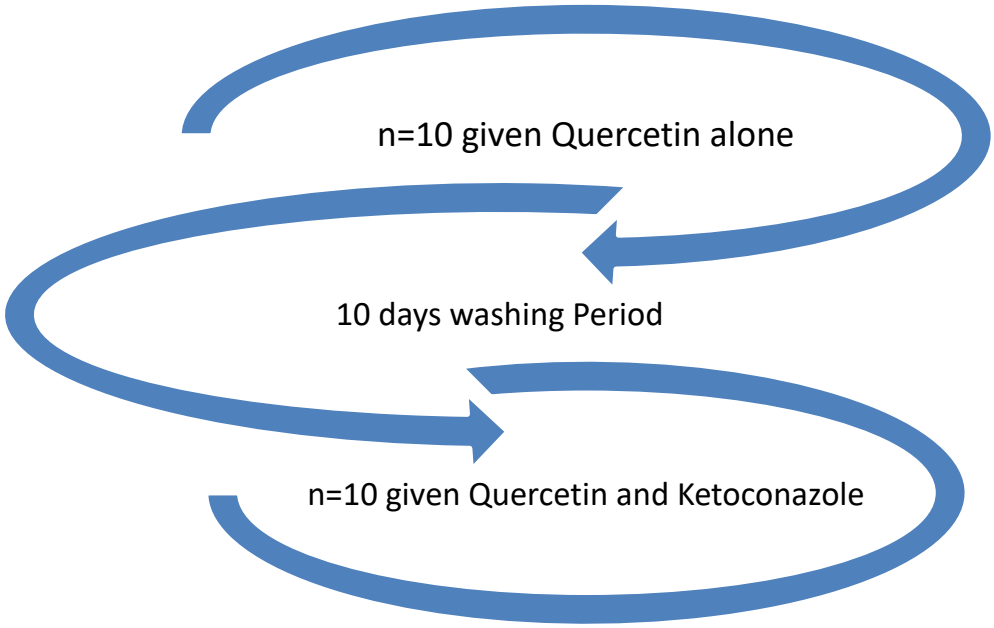

Figure 1: Study Design of the experiment.

10 apparently healthy adult volunteers, 5 male and 5 female have participated into the study. With average age of 23.91 ± 0.77 years old for the whole participants and 22.8 ± 0.84 for male and 23.6 ± 1.14 for female as other parameters are taken into account including weight, Hight and body mass index are shown in Table 3. A biochemical analysis was done before the study to assess the health for all individual involved in the study as shown in Table 4 and Table 5.

Volunteers involved in the study twice with 10 days apart as a washing-out period to eliminate any interaction between the 2 experiments. One of experiment using 1000 mg Que alone and the other experiment KETO 400 mg is added, as shown in figure 1.

All medications are taken on empty stomach, standard meals are provided during this experiment 2 hr. after starting experiment and

The average age was (23.91 ± 1.25) years, the weight was 72.80 ± 2.39, Hight was 171.40 ± 2.50 cm while the body mass index (BMI) was 24.80 ± 1.25 kg/m2 the male to female ratio was 1:1 and there was no significant difference between the age of male and female involved in the study 22.80 ± 0.84 for male and 23.6 ± 1.14 for female as shown in table 3:

Table 3 Demographical Data for the participants:

	Age years	weight (kg)	Gender	Hight (cm)	BMI (kg/m2)
Volunteer 1	23	70	M	173	23.4
Volunteer 2	22	71	M	176	22.9
Volunteer 3	22	73	M	174	24.1
Volunteer 4	23	75	M	172	25.4
Volunteer 5	24	77	M	169	27
Volunteer 6	24	72	F	168	25.5

Volunteer 7	25	71	F	169	24.9
Volunteer 8	24	74	F	170	25.6
Volunteer 9	23	70	F	172	23.7
Volunteer 10	22	75	F	171	25.6
Mean	23.2 ± 1.03	72.8 ± 2.39		171.4 ± 2.5	24.8 ± 1.25

Table 4 Laboratory parameters of the participants:

	Urea (mg/dL)	**Creatinin (mg/dL)**	**ALT (U/L)**	**AST (U/L)**	**Alkaline Phpsphatase (U/L)**	**Total bilirubin (mg/dL)**	**Direct Bilirubin (mg/dL)**	**Indirect Bilirubin (mg/dL)**
Volunteer 1	28.23	0.9	18.74	15.32	81.55	0.43	0.16	0.27
Volunteer 2	37.92	0.98	19.76	17.75	78.65	0.57	0.21	0.36
Volunteer 3	38.66	1.06	15.84	15.64	106.87	0.63	0.2	0.44
Volunteer 4	24.2	0.92	23.46	18.85	82.54	0.66	0.16	0.5
Volunteer 5	22.28	0.87	31.76	22.29	97.1	0.26	0.08	0.18
Volunteer 6	29.38	0.74	16.62	23.87	62.2	0.37	0.14	0.23
Volunteer 7	31.59	0.72	10.41	12.92	43.52	0.29	0.13	0.16
Volunteer 8	19.8	0.57	10.35	14.76	82.71	0.35	0.14	0.21
Volunteer 9	23.08	0.67	12.69	14.08	106.7	0.62	0.2	0.42
Volunteer 10	23.86	0.91	10.28	11.13	95.76	0.86	0.32	0.55
Mean	27.90 ±6.52	0.83 ± 0.15	16.99 ± 6.86	16.66 ± 4.05	83.76 ± 19.72	0.50 ± 0.19	0.17 ± 0.06	0.33 ± 0.14

Table 5 Laboratory parameters of the participants:

	Total Cholesterol (mg/dL)	TG (mg/dL)	LDL Cholesterol (mg/dL)	VLDL Cholesterol (mg/dL)	Non HDL Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)
Volunteer 1	155.98	103.38	106.64	30.68	108.38	47.6
Volunteer 2	132.06	144.39	78.72	50.88	92.84	39.22
Volunteer 3	119.31	115.24	73.52	33.05	81.71	37.61
Volunteer 4	139.3	120.19	79.5	84.04	99.62	39.67
Volunteer 5	123.98	116.6	74.87	33.32	85.08	38.9
Volunteer 6	141.66	48.31	73.71	13.52	71.07	70.59
Volunteer 7	144.85	53.96	80.2	10.79	76.63	68.22
Volunteer 8	79.76	59.83	29.23	11.97	27.54	52.22
Volunteer 9	117.14	94.2	68.76	18.84	66.98	50.16
Volunteer 10	159.15	70.88	88.85	14.18	88.7	70.46
Mean	131.32 ± 23.08	92.69 ± 32.75	75.4 ± 19.41	30.13 ± 22.89	79.86 ± 22.29	51.47 ± 13.59

Material And Method

Quercetin Standard ≥95% (HPLC), solid, purchased from Sigma Aldrich, USA and were used as received.

Quercetin capsule 500 mg, from We Like Vitamines ®, USA

Ketoconazole 200 mg, Nizoral® tablet from Janssen Cilag, Canada.

Methanol HPLC grade LiChrosolv® from Merk, Germany.

Phosphoric Acid 85%: PanReac ApplicChem ITW Reagents ®, Spain.

Deep Green, 18G I.V. canula 1.3x45cm 90ml/min, from Polymed ® Medical Devices, India.

EDTA K3 vacuum blood collection Tube ®, from AlHanoof for Medical and Lab supplies, Jordan.

1.5 mL Eppendorf's tube, from AlHanoof for Medical and Lab supplies, Jordan.

Cooling Centrifuge, from HERMLE labortechnik GmbH ®, Germany.

Sample Preparation

Samples collection

Venous blood samples of 5 ml was withdrawn using deep Green, 18G I.V. canula on the following intervals 0 hr., 0.5-hr., 1.5-hr., 2.5-hr., 3.5-hr., 4.5-hr., 5.5-hr., 6.5-hr., 7.5-hr., 8.5-hr. ,9.5-hr., 10.5-hr., 11.5-hr., 12.5-hr. and after 24 hr. each transferred to tube containing EDTA. (41)

Plasma Separation

Samples were centrifuged at 4°C , 3000 rpm for 10 minutes then the plasma collected by 1000 µL microdropper and stored in 1.5 mL Eppendorf's tube in deep freezer at —60°C until HPLC analysis. (42)

Preparation of samples for HPLC method.

The sample then thawed, and 0.5 mL of Methanol added to 1 mL of sample then and mix vigorously then centrifuged at 10000 rpm and -3°C for 15 minutes the proteins are precipitated and the sample ready for HPLC analysis.

HPLC

Column: 250x4.6mm Orbit 100 C18 5µm

HPLC Device and: LC100

Software: HPLC ver. 1.06 Copyright © 2005

Methanol HPLC grade LiChrosolv® Merk, Germany.

Wavelength: 370nm

Mobile Phase: Methanol 80: Water 19: phosphoric Acid 1

Calibration Curve preparation

Standard stock solution prepared using Que 25 mg dissolved in Ethanol 50 ml to get 0.5 mg/ml of the stock solution which is then undergo 10 times dilution get 50 µg/ml of it then serial dilution prepared to the following concentration 0.04 mg/ml, 0.035 mg/ml, 0.03 mg/ml, 0.025 mg/ml, 0.02 mg/ml, 0.015 mg/ml. the absorbance of these concentrations is taken using HPLC to prepare the calibration curve (Figure 1)

Table 1 Concentration and Absorbance of Standard prepared to prepare the calibration curve.

Concentration (µg/ml)	40	35	30	25	20	15
Absorbance (mAu)	285.0	260.3	216.7	176.0	142.7	104.3
Equation	y=7.202x+0.004				R^2 = 0.9996	

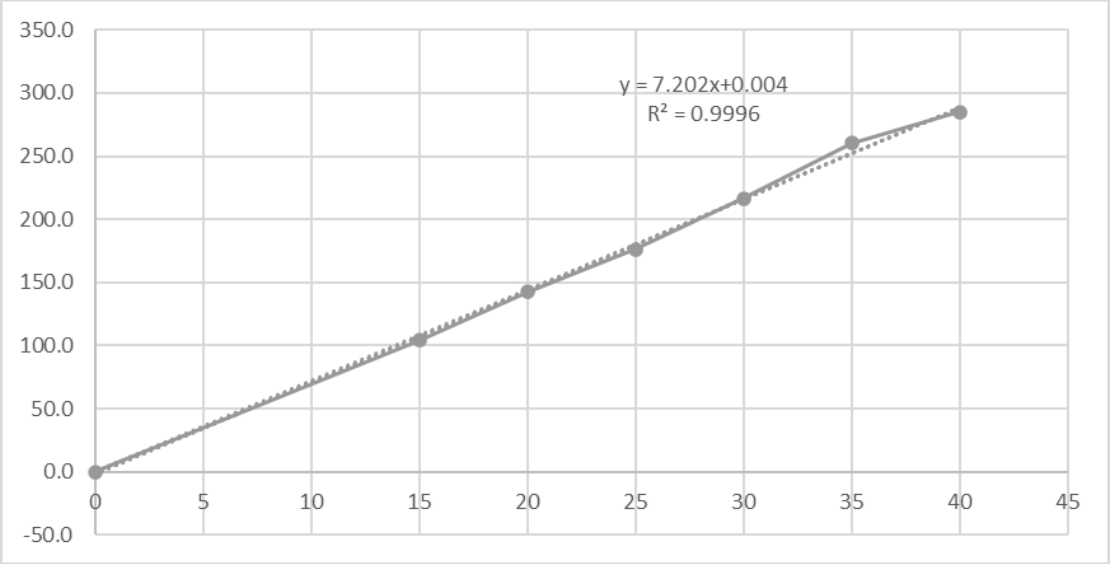

Figure 2: Calibration Curve

Concentration time curve presentation

Data from each participant from each group was represented as a concentration time curve to discriminate whether single or two compartment model and the order of elimination kinetic. (43)

Data Curve Fitting

The data curve fitting was done for mean plasma concentration data of free unchanged Que after oral administration for Que alone and Que and KETO group. Nonlinear regression analysis was performed using GraphPad Prism software 2019 (GraphPad Software Inc., San Diego, CA). Mean residuals and regression coefficient were calculated for free unconjugated Que as concentration (C) and for natural logarithm of concentration (ln C) and reciprocal of the concentration (1/C). both of data are fit to first order kinetic and linear equation was obtained for elimination phase of absorption and distribution.

Concentration time curve shows better fitting for 1st order kinetic, elimination phase with R value for fitting as in table 2.

Table 2 Goodness of fit table for elimination phase. Data expressed as Mean ± Standard deviation for natural logarithm of concentration

Study Groups	**R^2**	**SS (Sum squares of residuals)**	**P value**
Que Alone	0.9509	41.64	<0.0001
Que & KETO	0.9724	8.477	0.0001
For data fitting, best fitting is the one with R^2 nearest to 1 and minimum SS			

Mean and SD values were calculated by Microsoft Excel (Microsoft Co.). Pharmacokinetic parameters, including maximum plasma concentration (Cmax), observed time of maximum plasma concentration (Tmax), elimination t1/2 and area under the plasma concentration versus time curve (AUC).

Determination of AUC:

AUC was determined from concentration time curve from time zero to the final measurable concentration AUC (0-9.5h), using the linear trapezoidal method, Then, using the terminal elimination rate constant Ke (calculated by log-linear regression of the terminal portion of the plasma concentration-time curves), extrapolate to infinity. The linear trapezoidal method was also used to calculate total AUC.

Determination of Cmax and Tmax

Cmax and Tmax for participtant was found by using Microsoft Excel and visual inspections of the curves of plasma concentration-time, the equation of 0.693/Ke was used to calculate T½.

Elimination rate constant (Ke) values were calculated from regression analysis for each individual participant by finding slope from the terminal of the curve.

Statistical analysis

Was performed twice using Microsoft excel data analysis tool kit, and GraphPad Prism software 2019, t test was used to compare the results between two groups, the data expressed as mean± standard deviation. and p value <0.05 was considered significant

Results

AUC

AUC including AUC (0-24.5hr), AUC (24.5-∞hr) and AUC Total for each volunteer in each group was calculated then the average of these data was found in each group.

The AUC (0-24.5hr) significantly greater in Que - KETO group vs Que alone group (323.6 ± 55.58 vs 228.51 ± 28.67 respectively, P value <0.001), AUC (24.5-∞ hr) in Que - KETO group vs Que alone group (79.81 ± 41.02 vs 0 ± 0 respectively, P value <0.001), the total AUC was significantly greater in Que - KETO group as compared to Que alone group (403.41 ± 62.28 vs 228.51 ± 28.67, P value < 0.001) respectively.

Cmax and Tmax

While Cmax in Que group was significantly higher rather than Que - KETO group (34.73 ± 4.94, 29.85 ± 4.45 respectively P value = 0.0324), there was no significant difference in Tmax between the two groups with a result of (2.9 ± 0.7 hr. and 2.6 ± 0.32 hr. respectively P value = 0.239).

Ke, t1/2 and the time to complete elimination

The Elimination Constant (Ke) was (3.37 ± 0.58 hr.-1 and 0.08 ± 0.02 hr.-1 P value 0.000) in Que group and Que and KETO group while

t1/2 was higher in Que and KETO group than Que alone group with a result of (9.19 ± 2.19 hr. and 4.11 ± 0.61 hr. P value 0.000) respectively, and

the time to complete elimination was also longer in Que and KETO group than Que alone group with a result of (44.66 ± 9.17 hr. vs 12.93 ± 1.4 hr. P value 0.000) respectively.

When given together, Que and KETO were well tolerated. During the experiment, there were no major adverse events or clinically significant changes in clinical laboratory values, vital signs, or medical exams.

Table 6 Shows summary kinetic data for different study groups. Data expressed as Mean ± Standard deviation

Parameter	N	Unit	Que Alone	Que & KETO	P value
AUC (0-24h)	10	µg /ml.hr.	228.51 ± 28.67	323.6 ± 55.58	0.0001
AUC (24-∞h)	10	µg /ml.hr.	0.0 ± 0.0	79.81 ± 41.02	0.0002
AUC Total (0-∞h)	10	µg /ml.hr.	228.51 ± 28.67	403.41 ± 62.28	0.0001
C_{max}	10	µg/ml	34.73 ± 4.94	29.85 ± 4.45	0.0324
t_{max}	10	hr.	2.9 ± 0.7	2.6 ± 0.32	**0.2390**
K_a	10	h^{-1}	1.15 ± 0.06	1.12 ± 0.07	**0.2442**
K_d	10	h^{-1}	0.16 ± 0.12	0.1 ± 0.11	**0.3260**
K_e	10	h^{-1}	3.37 ± 0.58	0.08 ± 0.02	<0.0001
$t_{1/2}$	10	hr.	4.11 ± 0.61	9.19 ± 2.19	<0.0001
Time to complete elimination	10	hr.	12.93 ± 1.4	44.66 ± 9.17	<0.0001
P value <0.05% consider significant					

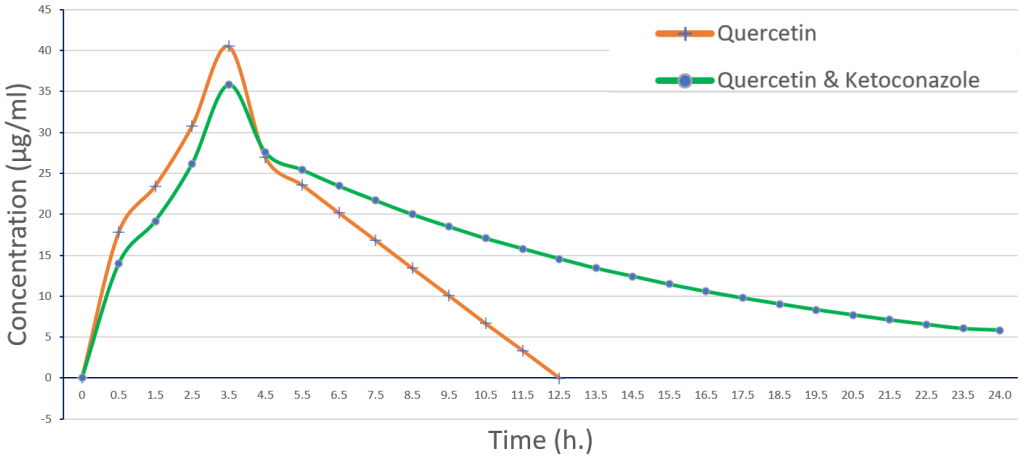

Figure 3: Concentration time Curve for mean plasma free unconjugated-Que in Que and Que +KETO groups of volunteers after fitting.

Quercetin green line with round sign,

Quercetins + KETO orange line with +

Discussion

Que is a dietary supplement flavonoid of plant origin, having substantial pharmacological effects and intriguing therapeutic potential for many clinical conditions since it has anti-oxidant, anti-diabetes, anti-inflammation, anti-viral ,anti-cancer, and anti-proliferation. (44, 45) Pharmacokinetically, After oral ingestion, Que is rapidly absorbed and has a bioavailability of roughly

2%. (25) Peak plasma concentrations are reached 1–2 hr. in normal volunteers, and the plasma t1/2 about 0.8 – 2.4 hr. (26, 27) Therefore, Many clinical studies of Que and its analogs have been conducted to improve its bioavailability. (46)

The results in this trial suggest that Que metabolism was reduced by KETO and its elimination phases was prolonged (increased Ke, t1/2 and time to complete elimination). The increased AUC of Que after KETO treatment was most likely related to a decrease in liver metabolism rather than a decrease in gut wall metabolism, as the Tmax of Que was insignificantly affected by KETO. (47)

KETO has pleiotropic inhibitory activity is evident from the fact that several P450- and non-P450-dependent enzymes including uridine 5′-diphospho-glucuronosyl-transferase (UGT) are inhibited. (48)

Coadministration of KETO reduced the Cmax and of Que this might be due to effect on P-glycoprotein (49, 50) or other factors affected by KETO like gut enzymes or carriers may be required for rapid Que absorption. (51)

Most of the Que is rapidly metabolized in the small intestine and liver by biotransformation enzymes into methylated, sulfo-substituted and glucuronidated forms, (52) by catechol-O-methyltransferase (COMT), sulfotransferase (SULT) and uridine 5′-diphospho-glucuronosyltransferase (UGT), respectively. (53)

Que metabolite are more water soluble with minimum or no biological activity (54) at the same time there is rapid disappearance or clearance of unconjugated (free) Que, (55) this was consistent with our results where free Que shows a t1/2 of around 4 hr. and this agreed with Moon et. al 2-4 hr. (6) and Terao et. al 4 hr. (28)

Addition of KETO which is well-known broad-spectrum enzyme inhibitor (56) significantly affect the metabolic deactivation of Que that appears clearly by reduce the rate of disappearance (ke) of Que form circulation which is reflected as elongation of t1/2 and the time to complete elimination as compared with Que, alone. (57)

The effect of KETO on Que elimination on human volunteers was never reported elsewhere, the AUC increase by co-administration of KETO in this study generally due to effect of KETO on the metabolic deactivation of Que by intestinal and liver enzymes, the effect of KETO was clear on the elimination phase of Que, where Ke was smaller than Ke of Que alone, consistent with this increase in exposure, effective t1/2 of Que was prolonged by coadministration of KETO, which might be more appropriate indicator of Que elimination, and finally the expected time required to complete elimination was extended to about 45 hr. as compared to about 13 hr. for Que alone.

The net result of the effect of KETO on Que kinetic leading to increase AUC, t1/2 and the time to complete elimination through prolonging the elimination phase of Que.

KETO effect on Que resemble the effect of KETO on Alisikren and saxagliptin as reported by Vaidyanathan et.al., and Patel et. al., where AUC of alisikren and saxagliptin was increased by co-administration with KETO. (58, 59) While KETO coadminstration with Apixaban and will increase the Cmax and AUC of increased by 62% and 99% respectively for Apixaban, and by 87% and 89% respectively for Edoxaban. (60, 61) Co-administration of KETO with tamsulosin increased Cmax and AUC of tamsulosin by a factor of 2.20 and 2.80, respectively. The terminal t1/2 was slightly increased from 10.5 hr. to 11.8 hr. (62) And after pretreatment with KETO, the t1/2 of risperidone increased significantly by 28·03 ± 40·60%. The AUC0–∞ increased significantly by 66·54 ± 39·76%. while, the Cmax and Tmax of risperidone were not significantly changed, indicating that KETO had minimal effect on the absorption of risperidone. (63)

Conclusions

Addition of oral KETO 400 mg single dose will affect the pharmacokinetic parameters of unconjugated Que through reducing its rate of metabolism and prolong elimination phase leading to increasing the AUC, t1/2 and the time required to complete elimination.

Recommendation

Further study effect of KETO on Que metabolite.

Effect of co administration of single dose KETO and Que with multiple oral doses of Que.

It is important to ascertain the safety of the combination if used for long period of time.

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