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Research Article

LIQUORICE BEVERAGE EFFECT ON THE PHARMACOKINETIC PARAMETERS OF ATORVASTATIN, SIMVASTATIN, AND LOVASTATIN BY LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY/MASS SPECTROSCOPY

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ABSTRACT

Objective: The objective of this study is to examine the effects of pre-consumption of freshly prepared liquorice beverage (4 ml/kg) on the pharmacokinetic (PK) parameters of (80 mg/kg) oral dose of atorvastatin, simvastatin, and lovastatin in healthy rats plasma.

Methods: A simple, rapid, and applicable analytical method was developed for the determination of each statin in rats' plasma. This method uses liquid chromatography-mass spectroscopy/mass spectroscopy. The mobile phase composed of methanol and formic acid in water and glimepiride as an internal standard. 108 rats were used in this study. Liquorice juice was given, and then each of the statins was given to test groups and liquorice only to the control groups, and then plasma samples were withdrawn on specific time schedule then PK analysis was performed.

Results: The analytical method showed acceptable linearity, recovery, precision, and accuracy. Administration of liquorice resulted in a significant increase in maximum concentration in plasma (C_{max}) of the three statins, also the area under plasma level-time curves (area under curve) was increased significantly. Moreover, the bioavailability of the drugs. On the other hand, the elimination of the three drugs showed no great changes, which suggests an interaction between liquorice and the transporting system of statins on the gut and biliary wall.

Conclusion: Consumption of liquorice results in increase bioavailability of atorvastatin, simvastatin, and lovastatin.

Keywords: Liquorice, Atorvastatin, Liquid chromatography-mass spectroscopy/mass spectroscopy, Simvastatin, Lovastatin, Pharmacokinetic parameters.

INTRODUCTION

Atorvastatin, simvastatin, and lovastatin are drugs that belong to a group named statins. Statins lower plasma levels of lipid by decreasing endogenous cholesterol synthesis via inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in the liver. This enzyme catalyzes the conversion of HMG-CoA to mevalonate; an early and rate-limiting step in cholesterol biosynthesis [1,2].

Statins are widely used in many countries for the treatment of both severe familial and non-familial hypercholesterolemia [3,4]. Hypercholesterolemia is known as the imbalance of blood lipids that is considered as a high-risk factor for inducing atherosclerosis and cardiovascular diseases which are now considered one of the most leading causes of death over the world [5,6].

The pharmacokinetics (PK) of individual statin drugs is affected by their lipophilicity. The more hydrophilic compounds, such as atorvastatin, are actively transported into the liver and are less metabolized by the cytochrome P450 (CYP-450) family, and exhibit more pronounced active renal excretion, while the less hydrophilic compounds such as simvastatin and lovastatin may be transported partially by passive diffusion and are better substrates for both CYP enzymes and transporters involved in biliary excretion. Simvastatin and lovastatin are both given as lactone prodrugs and converted to the active beta-hydroxy form, and the mechanism for this does not appear to be via CYP pathways [7].

Atorvastatin is highly soluble and is completely absorbed after oral administration. However, atorvastatin suffers extensive first-pass metabolism in the gut wall as well as in the liver which results in low bioavailability (14%). The volume of distribution of atorvastatin acid is 381L, and plasma protein binding exceeds 98%. Atorvastatin is extensively metabolized in both the gut and liver by oxidation, lactonization, and glucuronidation; and the metabolites are eliminated by biliary secretion and direct secretion from blood to the intestine. *In vitro*, atorvastatin is a substrate for P-glycoprotein (P-gp), organic anion-transporting polypeptide C, and H^+-monocarboxylic acid co-transporter. The total plasma clearance of atorvastatin is 625 mL/min and the half-life is about 7 hrs. The renal route is of minor importance (<1%) for the elimination of atorvastatin. *In vivo*, CYP-450 3A4 is responsible for the formation of two active metabolites from the acid and the lactone forms of atorvastatin [8].

Simvastatin is the methyl analogue of lovastatin. It is well-absorbed from the gastrointestinal tract but is highly extracted by the liver, and only 7% of the dose reaches the general circulation intact. Simvastatin is eliminated mainly in the feces due to biliary excretion, but only a small percentage of the dose is found in the stool in the form of the parent compound or simvastatin acid. Since simvastatin is metabolized by the CYP-450 system, a potential for drug interactions exists [9].

Many previous studies have shown that the effect and activity of any drug may differ from the expected findings as many drugs are exposed to drug-drug or food/beverage-drug interaction [10]. Recent published data have proven the existence of an intrinsic interaction between many popular juices such as grapefruit juice, orange juice, pomegranate juice, and drugs. These juices can alter the metabolism of drugs, which mostly causes alterations in PK and/or pharmacodynamics (PD) of drugs. The interaction could also be on the absorption level, which results in altering oral bioavailability, or through induction, or inhibition of metabolizing enzymes in gut or liver. These effects would be indicated by changes in plasma concentration of drugs [11-13].

Aqueous liquorice root extract is a popular traditional juice which is used worldwide especially in eastern countries; liquorice extract is used as a multi-component and multi-target agent as it may exert a holistic treatment to multi-factorial diseases [14,15].

A number of components have been isolated from licorice, including a water-soluble, biologically active complex that accounts for 40-50% of total dry material weight. This complex is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances. However, the main component is glycyrrhizic acid [16].

After oral administration of licorice in humans, the main constituent, glycyrrhizic acid, is hydrolyzed to glycyrrhetic acid by intestinal bacteria possessing a specialized ß-glucuronidase. Glycyrrhetic acid is rapidly absorbed and transported via carrier molecules to the liver. In the liver, it is metabolized to glucuronide and sulfate conjugates, which are subsequently rehydrolyzed to glycyrrhetic acid. Later is then reabsorbed, resulting in a significant delay in terminal clearance from plasma [16-18].

Administration of statins with fruit juices can alter their PK parameters since some juices inhibit CYP450 while others induce it. Those juices components might also interfere with P-gp transporters of influx and efflux mechanism [19,20].

This makes it important to study the possible interference of these beverages with drugs for the possible serious interactions.

The aim of this research is to develop and validate a simple and simultaneous analytical method for determining of atorvastatin, simvastatin, and lovastatin in rats plasma using liquid chromatography-mass spectrometry (LC-MS/MS), and to study the effect of liquorice fresh beverage consumption on the PK profile of these three statins in rats' plasma.

METHODS

Materials

Reagents

Deionized water, Nanopure (Fisher Scientific), rats plasma, (Harvested from Rats of University of Petra Animal House), methanol advanced gradient grade (Fisher Scientific), formic acid (GPR Rectapur), acetonitrile (ACN) (Fisher Scientific). Atorvastatin, simvastatin, lovastatin, and glimepiride raw materials (a gift from Dar Al-Dawa Pharma), crushed liquorices root (purchased from a local market).

Instrumentation

An API-MS was used. It is composed of the following: An on-line vacuum degasser (Agilent 1200) with solvent delivery systems pump (Agilent 1200), an auto-sampler (Agilent 1200), and a thermostat column compartment (Agilent 1200). API 4000 Mass Spectrometer was used with ACE 5, C8 (50 × 2.1 mm, 5 µm) column. A computer with a Windows XP system and SP3 Data Management Software 1.5.2 was used also. Another instrument is the Bath Sonicator Crest model-175T (Ultra Sonics CORP). Finally, Sartorius balance BP 2215 and a Centrifuge (Eppendorf 5417C) were used in the analysis procedure.

Animals

108 Sprague–Dawley (SD) adult male rats were supplied by the animal house of University of Petra with a weight range of 200-280 g. The rats were placed in an air-conditioned environment (20-25°C) and exposed to a photoperiod cycle (12 hrs light/12 hs dark) daily. All animal experiments were performed in compliance with the Federation of European Laboratory Animal Science Association guidelines, and the study protocol was approved by the Research Committee (September 2013) at the Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan.

Methods

Experimental procedures

To find the effect of fresh liquorice juice on three statins, fresh preparations of the drugs' solutions as well as liquorice beverage were carried out. All rats were fasted 1 day ahead of the statins administration. The rats received a specific dose orally by oral gavage according to their weights. The total number of rats which was 108 was divided into 3 groups. Each of which contains 36 rats. Then, each group was divided into two subgroups each containing 18 rats. The first group was administrated atorvastatin, the second; lovastatin, and the third group was fed with simvastatin (80 mg/kg). These groups were given water for drinking. The second subgroup in each group was given (4 ml/kg) of liquorice juice once daily for 3 consecutive days prior the experiment day, and then the statin (80 mg/kg) was given in the morning of the day of the experiment after 12 hrs fasting.

Following drug administration, few drops of rat's blood were withdrawn from the tail after 1, 1:30, 2, 2:30, 4, 6, and 8 hours and kept in eppendorf tubes. Each blood sample was centrifuged for 5-10 minutes to obtain the plasma volume of (100-125 µl), and then stored in a freezer at (−20C°) until the time of analysis.

Preparation of atorvastatin, simvastatin, and lovastatin solution to be given orally for rats

A sample with a weight of 0.3 g from each statin raw material was dissolved in 10 ml methanol. 1 ml was taken and completed with distilled water to 100 ml. The final obtained solution was with 0.3 mg/ml concentration.

Preparation of liquorice beverage

Fresh liquorice beverage was prepared by maceration. The strainer was filled with 250 g of plant material in 2 l of distilled cold water, left for 2 hrs then filtered to get the desired beverage. The volume then was completed to 2 L to produce 0.12 g/ml liquorice solution.

Preparation of stock and work solutions for the analytical method validation

A sample of each statin with a weight equivalent to 10 mg was dissolved in 200 ml of methanol to obtain a stock solution of 50 µg/ml concentration.

While the glimepiride (internal standard) stock solution was prepared by dissolving 10 mg in 100 ml of ACN to get a concentration of 100 µg/ml stock solution.

Then, the working solution was prepared by diluting 10 µl of the stock solution to 200 ml volume by ACN to produce a final concentration of 5.0 ng/ml of glimepiride.

Mobile phase

The mobile phase consists of an aqueous solvent of formic acid as buffer (35% of the mobile phase is 0.25% formic acid in water mixture) and an organic solvent; methanol (65%of the mobile phase). All the chromatographic and mass detector conditions are shown in Table 1.

Preparation of atorvastatin, simvastatin, and lovastatin standard calibration curve serial dilutions

Samples of the standard curve in plasma were prepared by spiking 20 µl from serial solution into 1 ml of plasma, using seven concentrations; not including zero, to obtain statins standard calibration curve concentrations of 0.500, 1.00, 2.00, 5.00, 8.00, 12.00, and 18.00 (ng/ml) in plasma. Each concentration of the plasma sample was divided into 50.0 µl in 150.0 ml eppendorf tube and kept at (−20°C) for the analysis as shown in Table 1.

Preparation of atorvastatin, simvastatin, and lovastatin quality control (QC) concentration solutions

QC samples in plasma were prepared by spiking 20 µl from serial solution into 1 ml of plasma to obtain QC concentrations of 1.5, 7.5, and

14.0 µg/ml. All QC plasma samples were kept at (−20°C) as shown in Table 2.

Method of extraction

Experimental procedure was as follows: 50.0 µl aliquots of each test sample (blank, zero, standards, QC low (QCL), QC mid (QCM), QC high (QCH), or Rat samples) were mixed with 150.0 µl of internal standard (5 ng/ml glimepiride), vortexed vigorously for 1.0 minutes then centrifuged at 14000 rpm for 15 minutes. Table 3 shows serial spiking samples in plasma.

Analytical method validation

Method validation was performed over 3 separate days. Seven standard calibration levels were prepared daily. The prepared plasma samples of method validation represented blank, zero, standard calibration curve, six replicates of QC samples, the lower limit of quantification (LLOQ), QCL, QCM, and QCH. The validation parameters; linearity, intra- and inter-day accuracy and precision, sensitivity, and recovery should be within the expected limits by the Food and Drug Administration (FDA) Guidance for Industry and United state pharmacopeia [21-23].

PK model building

Compartmental analysis

Simultaneous data fitting of the mean of results in each set of plasma data was done using Kinetica® program version 4.1. Plasma profiles were best characterized by (1) Compartmental model for lovastatin, (2) compartment model for simvastatin, and (3) compartment model for atorvastatin. The criteria used for model building involved

Table 1: Summary of LC-MS/MS conditions and results

Pump flow rate	1.0 ml/min						
Autosampler injection volume	5 µl						
Autosampler temp.	4°C						
Column oven temp.	40°C						
Mobile phase	Mixture of (65% methanol, 35% [0.25%] formic acid)						
Column type	ACE 5 C8 column (50×2.1 mm, 5 µ)						
Lovastatin retention time (minutes)	0.2						
Simvastatin retention time	0.21						
Atorvastatin	0.24						
Glimepiride (I.S)	0.3						
Analytes	**Q1 Mass**	**Q3 Mass**	**Dwell**	**DP**	**EP**	**CE**	**CXP**
MRM detection conditions							
Atorvastatin	500.605	440.1	150	81	9.5	30	30
Simvastatin	551.4	449.8	146	70	9.9	35	33
Lovastatin	577.2	443.7	148	88	9	33	36
Glimepiride (I.S)	496.47	351.9	149	90	10	20	21
MS conditions							
CUR	30						
CAD	10						
IS	5000						
TEM	500						

MRM: Multiple reaction monitoring, MS: Mass spectroscopy, CUR: Curtain gas, CAD: Collision gas, IS: Internal standard, TEM: Temperature, DP: Declustering potential, EP: Entrance potential, CE: Collision energy, CXP: Collision cell exit potential

Table 2: Preparation of atorvastatin, simvastatin, and lovastatin quality control samples in plasma

Solution no.	Serial solution of atorvastatin, simvastatin, and lovastatin from working solution of 50 µg/ml				Plasma spiking solution			
	Working solution concentration (µg/ml)	Stock concentration (µg/ml)	Volume taken from stock (µl)	Total volume (ml)	Cal ID	Volume taken from w.s (µl)	Total volume (ml)	Final concentration (ng/ml)
1	0.075	50	15	10	QCL	20	1	1.5
2	0.375	50	75	10	QCM	20	1	7.5
3	0.700	50	140	10	QCH	20	1	14.0

Table 3: Preparation of atorvastatin, simvastatin, and lovastatin serial spiking samples in plasma

Solution no.	Serial solution of atorvastatin, simvastatin, and lovastatin from working solution of 50 µg/m				Plasma spiking solutions			
	Working solution concentration (µ/ml)	Stock concentration (µ/ml)	Volume taken from stock (µl)	Total volume (ml)	Cal ID	Volume taken from W.S (µl)	Total volume (ml)	Final concentration (ng/ml)
1	0.025	50	5	10	S1	20	1	0.5
2	0.05	50	10	10	S2	20	1	1
3	0.1	50	20	10	S3	20	1	2
4	0.25	50	50	10	S4	20	1	5
5	0.4	50	80	10	S5	20	1	8
6	0.6	50	120	10	S6	20	1	12
7	0.9	50	180	10	S7	20	1	18

the examination of the fitted curves, the improvement in objective function and statistical t-test to examine if the differences between each parameter for drug alone and the drug given after liquorice was significant or not, using a 5% confidence interval. The calculated parameters are: Elimination rate constant (Kel), distribution rate constant (K_{12}), elimination half-life ($t_{1/2}$), the volume of distribution (V), and clearance (Cl).

Non-compartmental analysis

Non-compartmental analysis was performed for each plasma data to obtain the parameters: Elimination rate constant (Kel), area under curve ($AUC_{(0-t)}$), $AUC_{(0-\infty)}$, time to reach maximum plasma concentration (T_{max}) and maximum plasma concentration (C_{max}), area under first moment curve ($AUMC_{(0-t)}$), and mean residence time ($MRT_{(0-t)}$) using same version of Kinetica®.

RESULTS AND DISCUSSION

Validation

Validation of this analytical method was performed to be evaluated in terms of recovery, the linearity of response, precision, accuracy, and sensitivity for quantification of atorvastatin, simvastatin, and lovastatin. Inter-day and intra-day precision showed acceptable values. All coefficients of variation for LLOQ, QCL, QCM, and QCH were <15%.

On the other hand, inter-day and intra-day accuracy showed reasonable values, as mean calculated concentrations were within ±15% of nominated concentration for LLOQ, QCL, QCM, and QCH. Intra-day accuracy data showed an accuracy range of 99.7%-120.9%, 100.06%-110.00%, 99.84%-105.08% for the first, second, and 3rd day of the validation for the three statins. The accuracy for all statins overall the 3 days of validation was within the accepted criteria. Linear relation (R^2) performance ranged between 0.997 and 1. Results are shown in Tables 1 and 2. The plot of linearity of calibration curve levels for statins versus their analytical response and regression linear equation and the mean of the AUC ratio for each standard point is shown in Fig. 1.

Several plant-derived beverages have been shown to modulate enzymes and transporters in the intestine and liver, leading to altered PK and potentially negative PD outcomes. Commonly consumed fruit beverages mainly orange, grapefruit, liquorice, pomegranate, tea, and alcoholic drinks contain phytochemicals that inhibit intestinal CYP-450 and Phase II conjugation enzymes, as well as uptake and efflux transport proteins [21-23].

The concentration of statins in plasma is determined by the oral bioavailability of drug which involves the extent and rate of drug absorption. Furthermore, it depends on the first-pass effect which is determined by the activity of microsomal enzymes involved in the metabolism and the transport system of the biliary route of clearance which is the major route of clearance of statins [24-31].

The effect of liquorice on the statins PK parameters was expressed as a significant increase in maximum plasma level of the three statins and the area under plasma level-time curves (AUC) ($p<0.05$). Atorvastatin C_{max} elivated from 25.5±13.77 ng/ml to 31.80±19.23 in the presence of liquorice and the $AUC_{(0-last)}$ from 63.6 to 77.45 ng.hr/ml. For simvastatin, C_{max} was increased from 28.6±14.99 to 80.4±12.76 ng/ml and $AUC_{(0-last)}$ from 114.25 ng/ml to 285.25 ng.hr/ml, while lovastatin C_{max} increased from 32.40±10.93 to 93.3±11.22 ng/m and $AUC_{(0-last)}$ from 140.25 to 409.5 ng.hr/ml. The overall elimination pattern for the three drugs showed no big changes. Figs. 2-4 and Tables 4 and 5 show the kinetic parameters of compartmental and non-compartmental analysis.

In addition, the distribution rate from the first compartment (plasma) to the second compartment was almost the same (non-significant $p>0.05$), as well as the volume of distribution and MRT of the drug in the body from time zero up to eight hours with and without liquorice. The total body clearance with and without liquorice was also very close.

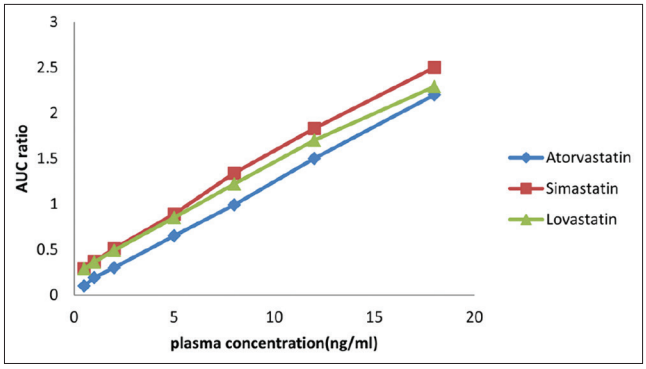

Fig. 1: The plot of linearity calibration curve levels for atorvastatin (r^2 = 0.997), simvastatin (r^2 = 0.996), and lovastatin (r^2 = 0.997) in rat plasma

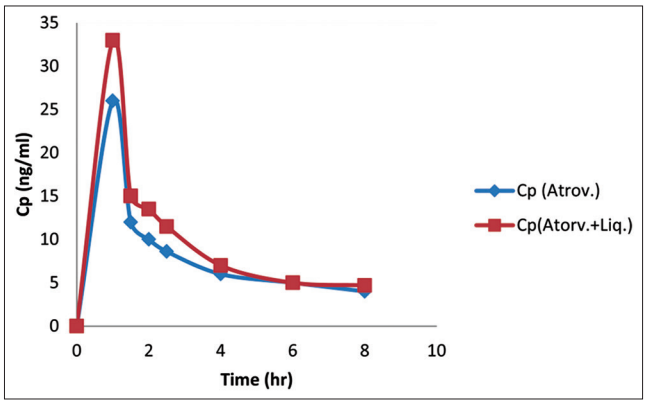

Fig. 2: Average rat plasma concentration of atorvastatin (atorv.) (ng/ml) versus time (hrs) profile showing the changes in mean plasma atorvastatin concentrations in presence and absence of liquorice (Liq.) juice (n=18)

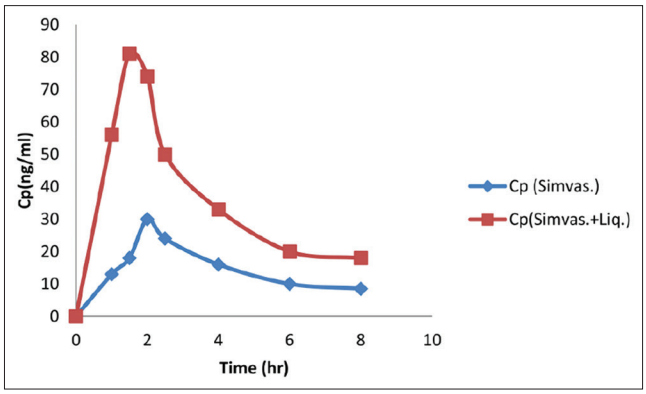

Fig. 3: Average rat plasma concentration (Cp) of simvastatin (Simvas.) (ng/ml) versus time (hrs) profile showing the changes in mean plasma simvastatin concentrations in presence and absence of liquorice (Liq.) juice (n=18)

These results suggest an increase in the amount of atorvastatin reached to the general circulation without any noticeable change in time course of drug absorption or elimination.

Regarding simvastatin, almost the same pattern of movement was observed with and without liquorice. However, in this case, the increase in C_{max} was highly significant which might result in an increase in distribution rate as a result of first-order distribution process accompanied by a slight decrease in time to achieve a maximum

Table 4: Results of compartmental analysis of atorvastatin, simvastatin, and lovastatin with and without liquorice

Parameter	Atorvastatin	Atorvastatin+ liquorice	Simvastatin	Simvastatin+ liquorice	Lovastatin	Lovastatin+ liquorice
Kel (hr^{-1})	0.11±0.01	0.1±0.02	0.158±0.015	0.151±0.016	0.17±0.009	0.2±0.01*
K12 (hr^{-1})	0.95±0.02	0.90±0.025	0.28±0.028	0.48±0.061*	-	-
$t_{½elim}$ (hr)	6.3±0.5	6.93±0.4	4.38±0.039	4.5±0.6	4.07±0.52	3.465±0.31
V (L/kg)	4.54±0.3	4.9±0.4	11±0.38	11.5±0.29	6.5±0.42	6.8±0.60
Cl (L/kg/hr)	0.5±0.10	0.49±0.15	1.738±0.099	1.736±0.12	1.105±0.21	1.36±0.0.19*

Results are expressed as average±SD. *Significant (p<0.05), n=18 for all experiments, significant p<0.05, non-significant p>0.05, 95%CI

Tablet 5: Results of non-compartmental analysis

Parameter	Atorvastatin	Atorvastatin+ liquorice	Simvastatin	Simvastatin+ liquorice	Lovastatin	Lovastatin+ liquorice
Kel (hr^{-1})	0.1±0.01	0.09±0.091	0.21±0.12	0.22±0.11	0.18±0.01	0.21±0.02*
C_{max} (ng/ml)	25.5±13.77	31.80±19.23*	28.6±14.99	80.4±12.76*	32.40±10.93	93.3±11.22*
T_{max} (hr)	1	1	2	1.5	2.5	1.5*
$AUC_{(0-8)}$ (ng.hr/ml)	63.6±5.0	77.45±4.5*	114.25±6.0	285.25±8.5*	140.25±4.3	409.5±7.1*
$AUC_{(0-\infty)}$ (ng.hr/ml)	103.6±6.2	129.67±4.2*	154.72±7.1	367.07±8.3*	159.75±4.3	589.94±8.2*
$AUMC_{(0-8)}$ (ng.hr^2/ml)	194±3.5	224.85±5.3*	413.25±4.7	652.75±6.8*	616.8±4.6	1709±9.3*
$MRT_{(0-8)}$ (hr)	3.05±0.12	2.90±0.11	3.6±0.21	2.28±0.99*	4.4±0.13	4.17±0.11

Results are expressed as average±SD. *Significant (p<0.05), n=18 for all experiments, significant p<0.05, non-significant p>0.05, 95% CI

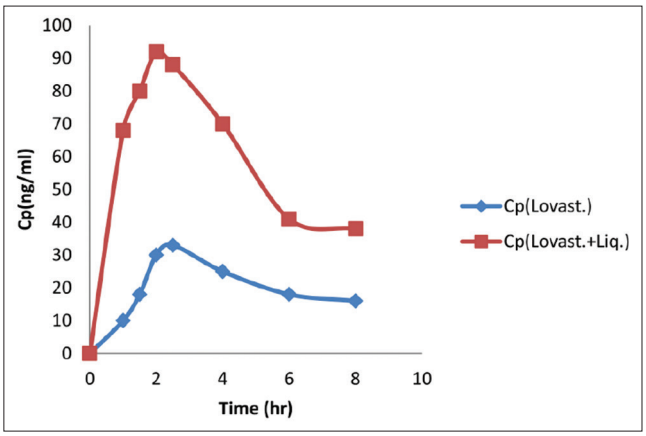

Fig. 4: Average rat plasma concentration (Cp) of lovastatin (Lovast.) versus time (hrs) profile showing the changes in mean plasma lovastatin concentrations in presence and absence of liquorice (Liq.) juice (n=18)

concentration in plasma. However, the elimination rate is almost the same, and the increase in $AUC_{(0-last)}$ and $AUC_{(0-\infty)}$ is highly significant.

In some studies conducted on human, lovastatin was reported to follow 2-compartmental model like simvastatin due to the high similarity in both structure and characteristics [32]. In this study, the best-fitted model is 1-compartmental model. The results indicate a high increase in C_{max} and AUC up to 8 hrs and infinity. T_{max} was shortened when the drug was given after liquorice and elimination half-life was slightly decreased. Statistically, the changes in Kel, $t_{½}$ and clearance are significant, but the overall time course kinetic of the drug does not seem much changed for an *in-vivo* study as indicated by the MRT.

Liquorice has been reported by many studies to have an effect on CYP enzymes in gut and liver. Some contents of liquorice have been showed to have an inhibitory effect on some CYP enzymes while others, an inducing one [33]. This is unlike grapefruit juice, which has been shown in many studies to have an inhibitory effect on the CYP family in general [34].

The interaction between liquorice with P-gp transporters both in the intestine and liver was also studied by many researchers [35-37]. Several statins antihyperlipidemic; including these used in this study, are known to be absorbed by both passive diffusion and P-gp transporters. Other studies have also reported an efflux mechanism for atorvastatin that partially participate in the low bioavailability of this drug [38]. Statins' major rout of clearance is via biliary clearance which depends mainly on active transport of statins from liver tissue to bile through these p-gp transporters. They also have very low bioavailability due to high biliary clearance in addition to minor conjugation pathway and the little amount by renal excretion. They also have very high plasma protein binding which makes free concentration in plasma sensitive to changes in any mechanism [31,39,40].

The results of this study suggest an increase in the bioavailability of the three drugs under examination. This could be attributed to the inhibitory effect of liquorice on the efflux mechanism in the GIT, which results in absorbing more of the drug to the portal vein. In addition, this increase in the bioavailability might also be due to the inhibitory effect or saturation of p-gp transporters on the biliary wall, which causes more drugs to escape from the liver to the general circulation. The magnitude of change in each drug parameters might depend on the specific transporter involved for each drug; its distribution and degree of interaction with liquorice components. Since there were no great changes in elimination half-lives, clearance, and total time residence of these drugs, the effect of liquorice on these three statins might be evident in their antihyperlipidemic activity as a result of the increase in plasma levels and intensity of action. Biochemical studies are suggested to evaluate the lipid profile after single and multiple doses of these statins when taken with liquorice.

CONCLUSION AND FUTURE WORK

A validated bioanalytical method with high resolution and sensitivity was developed for the determination of atorvastatin, simvastatin, and lovastatin levels in rats plasma. The effect of fresh liquorice beverage on the PK profile for three statins was shown to cause an increase in their bioavailability. This was expressed as higher concentrations in plasma and larger area under concentration-time profile, with no clear changes in the elimination patterns of the three drugs.

Further *in vitro* and *in vivo* investigations are suggested. This study can lead to many possible future studies such as the administration

of different quantities of liquorice juice to detect any dose-dependent changes in PK parameters. Other studies could also be extended to healthy and hyperlipidemic human.

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