Pomegranate and licorice juices modulate metformin pharmacokinetics in rats

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Abstract

OBJECTIVES: Food or drinks may significantly alter the pharmacokinetics and pharmacodynamics of drugs which may lead to adverse events. A drug such as metformin is widely used to regulate plasma glucose level and pomegranate and licorice have been identified to help in type-2 diabetes management. However, the interactions of the latter on metformin pharmacokinetics were not studied. Therefore, we aimed here to study the impact of pomegranate and licorice on metformin pharmacokinetics in rats.

METHODS: Juices were given to rats for two days and half an hour before metformin (20 mg/kg) oral administration. Blood samples, then, were collected at different time intervals, processed and analyzed using validated reliable HPLC method. Plasma profile and pharmacokinetic parameters were calculated for each group.

RESULTS AND CONCLUSION: Pre-administration of pomegranate significantly reduced metformin maximum plasma concentration from 1410 to 1031 ng/ml. On the other hand, pre-administration of licorice significantly delayed metformin reaching its maximum plasma concentration. In conclusion, pre-administration of pomegranate may potentially reduce efficacy of metformin while licorice might delay metformin action. Thus, both juices should be cautiously administrated with metformin, the mainstay drug for type-2 diabetes mellitus management.

INTRODUCTION

Studying the interactions of food on drugs are essential to be carried out. Such interaction studies evaluate drug appropriate dosing, timing, and formulation of new drug candidates (Won et al. 2012). In this regard, several juices have shown to alter enzymes and transporters that modulate the pharmacokinetic (PK) parameters and thus might result in undesired pharmacodynamic (PD) outcomes (Won et al. 2012).

The majority of the previously reported drug–juice interactions focused mainly on grapefruit juice. On the other hand, interactions with several other juices with drugs are generally unnoticed which still need to be investigated and addressed (Paine et al. 2004). Pomegranate juice (Punica granatum from the family-Lythraceae) has become...
highly recommended supplements as a natural antioxidant (Jarvis et al. 2010). However, its interaction with various drugs was previously addressed (Misaka et al. 2011). Recent studies proved that pomegranate inhibits CYP3A in the body (Hidaka et al. 2005). In addition, it was reported that pomegranate juice influenced the intestinal absorption of certain drugs through an effect on their transporter (Adukondalu et al. 2010). Licorice (Glycyrrhiza glabra of the Leguminosae Family), on the other hand, one of the well-known cultural drinks particularly in Middle East region. It is known for its anti-inflammatory, hypocholesterolemic and antioxidant effects (Hou et al. 2012). However, licorice was found to interact with some drugs’ kinetics. It was reported that licorice significantly reduced cyclosporine bioavailability through activating P-glycoprotein and CYP3A4 (Hou et al. 2012).

Metformin is a biguanide derivative drug and indicated for the management of type 2 diabetes mellitus (DM) mainly through reducing gluconeogenesis, glucose absorption, and enhancing glucose uptake (Howlett and Bailey 1999). Although, metformin is usually considered the first line for managing DM, it can be introduced as a single therapy, in combination with other oral anti-diabetic drugs or insulin (Setter et al. 2003; Nathan et al. 2009; Mazokopakis & Starakis 2012). Following an oral dose, metformin is completely absorbed within 6 hours from the upper intestinal part giving an oral bioavailability of 40–60% whereas its peak plasma concentration (Cmax) is reached after 2–3 hours and plasma elimination is between 2–6 hours (Scheen 1996).

Type-2 diabetes incidence is increasing dramatically worldwide and metformin is one of the first line drugs in hyperglycemia management. However, diabetic patients are recommended to ingest some natural food or drinks that may regulate their plasma glucose level. Some of this food or drinks may interact with the pharmacokinetics of co-administrated drugs resulting in either a positive or negative effect. In either case, the dose should be adjusted or such food or drink may be prohibited. In this regard, it has been shown that pomegranate and licorice juices may help in type-2 diabetes (Banihani et al. 2014; Sawada et al. 2014). However, the interaction of the latter juices on metformin pharmacokinetics were not studied. Therefore, we aimed here to study the impact of pomegranate and licorice on metformin pharmacokinetics in rats.

MATERIALS AND METHODS

Chemicals and reagents

Metformin and cefadroxil were of analytical purity grade and purchased from United Pharmaceuticals. 1-Hexanesulfonic Acid sodium Salt was purchased from (Chromanorm), potassium dihydrogen phosphate buffer was obtained from (Scharlau), trichloroacetic acid was obtained from (MERCK). Methanol, water, and acetonitrile were of HPLC grade (Chromanorm).

Pomegranate and licorice juices preparation

Pomegranate and licorice juices were freshly prepared a day before the day of an experiment, refrigerated at 4°C and supplied to rats as such without further treatment. Pomegranate fruits were purchased from a local market (Amman, Jordan). Pomegranate juice was freshly hand squeezed. Licorice root juice was prepared according to the traditional way by soaking in water for one hour, then the roots transferred to special perforated can usually supplied with the root. Cold water was then allowed to drizzle over it for 2 hours.

Animals

Male and female Sprague–Dawley rats (150–250 g) were housed in conditioned environment with 12 h light/dark cycles. Food and water were available ad libitum until 12 h prior to an experiment. Before an experiment, the food was removed and rats were kept fasting overnight. The experimental protocol had been approved by the ethics committee of the Research Council, Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan.

Study design

Metformin (20 mg/kg) was dissolved in distilled water and freshly prepared at the day of an experiment. Rats received a calculated volume by using a stainless steel oral gavage needle. Rats were randomly divided into three groups; metformin (9 rats), metformin and pomegranate (9 rats) and metformin and licorice (10 rats). Freshly prepared juices were given for 12 h in drinking water before the experiment and half hour before the dose of metformin, a booster dose (5 ml) of juice or water for metformin group was given. Blood samples were taken from the rats optical vein and placed into an EDTA-containing tubes at the following time points: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.0, 6.5 hours.

HPLC analysis

An HPLC (FINNIGAN SURVEYOR) was used and composed of the following: ChromQuest software 4.2.34 Solvent delivery systems pump (LC Pump Plus), autosampler Plus, UV-VIS Plus Detector, Hypersil Thermo Electron Corporation, BDS C-18 Column (150 mm × 4.6 mm, 5 μm) and computer System, Windows XP, SP3.

A mobile phase consisting of 90% of water contains 7.5 mM potassium dihydrogen phosphate buffer and 15 mM 1-hexanesulfonic acid, sodium salt and 10% of acetonitrile was circulated through a reversed-phase Thermo Scientific column (BDS HYPERSIL C 8) at flow rate of 0.9 ml/minute. Absorption was measured at 234 nm wavelength (Table 1). The sample extraction method was as follows: 150μl aliquot of each test sample (blank, zero, standards, quality control samples or rat samples) was added to 150μl of 5 μg/ml cefadroxil (Internal Standard), vortexed for 1 minute and then tubes were centrifuged at 14 000 rpm for 10 minutes.
(Table 1). A clear supernatant was transferred to a flat bottom insert and 50 μl was injected directly into HPLC system.

**Method validation**

Accuracy, precision and linearity tests were carried out according to EMEA guideline. Regarding linearity, seven calibration points (25 ng/ml, 50 ng/ml, 200 ng/ml, 500 ng/ml, 1000 ng/ml, 2000 ng/ml and 2500 ng/ml) were prepared and used. A series of six injections of each calibration concentration level were performed. Peak areas of the calibration standards were plotted in the Y-axis against the nominal standard concentration, and the linearity of the plotted curve was evaluated through evaluation of the correlation coefficient (R²) which was more than 0.99. The intra-day precision and accuracy were evaluated by analyzing six replicates of the quality control (QC) samples (low, mid, high) and lower limit of quantification (LLOQ) samples on a single day. The inter-day precision and accuracy were determined by analyzing three runs of QC samples and LLOQ samples on three different days. The accuracy (%) was calculated by dividing a measured mean concentration over the nominal concentration. Precision was presented as CV%. The acceptable values of accuracy and precision are below 15% except at the LLOQ, for which accuracy and precision should be below 20%.

**Data analysis**

Pharmacokinetics parameters were calculated by non-compartmental analysis (NCA) model using WinNonlin software V 5.2. The following parameters were estimated: Area under the curve to 6 hr (AUClast), the maximum concentration of drug in plasma (Cmax) and time to achieve Cmax (Tmax). The statistical analysis was assessed on the difference between Cmax, Tmax and AUClast between each 2 groups using the independent samples Student’s t-test. The p-value <0.05 is considered significant.

**RESULTS AND DISCUSSION**

**Validation results**

In order to demonstrate the reliability of our method for the determination of metformin in rat plasma, method validation according to EMEA guidelines was carried out. Using the defined chromatographic method, metformin and cefadroxil were separated within 6 minutes run time (Figure 1). The correlation coefficient (R²) for the calibration curve of metformin for each run was more than 0.99. Furthermore, the intra-day and the inter-day accuracy values of metformin were between (100.74–104.65%) and (100.99–103.75%), respectively, while the intra- and inter-day precision values were equal to or less than 4.62% and 4.42%, respectively (Table 2). As illustrated, the present assay provides reasonable accuracy, precision, linearity for metformin over the concentration range tested since all of the results were found within the acceptance criteria of validation guidelines.

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**Table 1. Chromatographic conditions of metformin HPLC assay.**

<table>
<thead>
<tr>
<th>HPLC Conditions</th>
<th>Pump Flow Rate</th>
<th>Column Oven Temp</th>
<th>Autosampler Temp</th>
<th>Autosampler Injection Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatography</td>
<td>0.9 ml/min</td>
<td>25 °C</td>
<td>10 °C</td>
<td>50 μl</td>
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<tr>
<td>Mobile phase</td>
<td>90% of water contains 7.5 mM potassium dihydrogen phosphate buffer and 15 mM 1-hexanesulfonic acid, sodium salt, 10% of acetonitrile. pH=3.80, adjust with H₃PO₄</td>
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<tr>
<td>Column type</td>
<td>Hypersil Thermo Electron Corporation, BDS C-8 Column (150 mm × 4.6 mm, 5μm)</td>
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<tr>
<td>Retention times (minutes)</td>
<td>Metformin</td>
<td>Cefadroxil (Internal Std)</td>
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<td></td>
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<tr>
<td></td>
<td>5.9</td>
<td>4.6</td>
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<tr>
<td>Detection conditions</td>
<td>Wavelength</td>
<td>234 nm</td>
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**Fig. 1.** Chromatograms of blank (A) and plasma sample containing metformin 2250 ng/ml and internal standard 5 μg/ml (B).
**Pomegranate reduces C_{max} of metformin**

Plasma profile of metformin and its pharmacokinetic parameters were illustrated in Figure 2 and Table 3, respectively. Metformin reached its C_{max} (1410 ng/ml) at 2 hr. However, pre-administration of pomegranate juice significantly reduced C_{max} of metformin (1031 ng/ml, 27%, *p<0.05). Although, the AUC_{last} of metformin was reduced and T_{max} was delayed when pomegranate was pre-administered, the differences did not reach significant levels (Table 3).

Since metformin is mainly excreted unchanged (Scheen 1996), the possible explanation for the reduction of metformin plasma level with pomegranate is due to pomegranate impact on the absorption of metformin. Accordingly, Adukondalu et al. (2010) found that the transport of carbamazepine in non-everted and everted sac methods was reduced by the treatment of pomegranate juice. In addition, it was found that pomegranate juice influenced the intestinal transport of buspirone in rats (Shravan Kumar et al. 2011). As metformin intestinal absorption mediated primarily by plasma membrane transporter, pomegranate may inhibit these transporters and thus inhibit or limit metformin absorption. (Dresser et al. 2002; Farkas and Greenblatt 2008)

**Licorice delays T_{max} of metformin**

Pre-administration of licorice did not alter AUC_{last} or C_{max} but it significantly delayed the T_{max} of metformin (2.3 h vs. 3.1 h; Fig. 2, Table 3). Previously, it has been shown that licorice decreases the clearance of prednisolone and increases prednisolone bioavailability by inhibiting its metabolism (Chen et al. 1991). In addition, Hou et al. found that licorice significantly reduced the oral bioavailability of cyclosporine through activating P-glycoprotein and CYP3A4 (Hou et al. 2012). In this study, licorice didn’t modulate the bioavailability of metformin since both C_{max} and AUC were not altered significantly. On the other hand, a significant shift in T_{max} was observed. Therefore, licorice may delay the absorption of metformin. A possible explanation might be related to the reversible effect of the juice on metformin plasma membrane transporters.

**CONCLUSION**

In conclusion, pomegranate significantly decreased plasma level of metformin. On the other hand, licorice causes significant delay in T_{max} of metformin. Thus, caution should be taken when these juices co-administrated with the mainstay medication of diabetes mellitus, metformin.

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**Competing interests**

The authors declare that they have no competing interests.
REFERENCES


