# 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 sample 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: 150 μl aliquot of each test sample (blank, zero, standards, quality control samples or rat samples) was added to 150 μl of 5 μg/ml cefadroxil (Internal Standard), vortexed for 1. minute and then tubes were centrifuged at 14 000 rpm for 10 minutes

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

| HPLC Conditions | Pump Flow Rate | Column Oven Temp | Autosampler Temp | Autosampler Injection Volume |
|---|---|---|---|---|
| | 0.9 ml/min | 25 °C | 10 °C | 50 µl |
| **Chromatography** | **Mobile phase** | 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 $H_3PO_4$ | | |
| | **Column type** | Hypersil Thermo Electron Corporation, BDS C-8 Column (150 mm × 4.6 mm, 5µm) | | |
| | **Retention times (minutes)** | Metformin | Cefadroxil (Internal Std) | |
| | | 5.9 | 4.6 | |
| **Detection conditions** | **Wavelength** | 234 nm | | |

(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, 1 000 ng/ml, 2 000 ng/ml and 2 500 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 ($R^2$) 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 ($AUC_{last}$), the maximum concentration of drug in plasma ($C_{max}$) and time to achieve $C_{max}$ ($T_{max}$). The statistical analysis was assessed on the difference between $C_{max}$, $T_{max}$ and $AUC_{last}$ 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
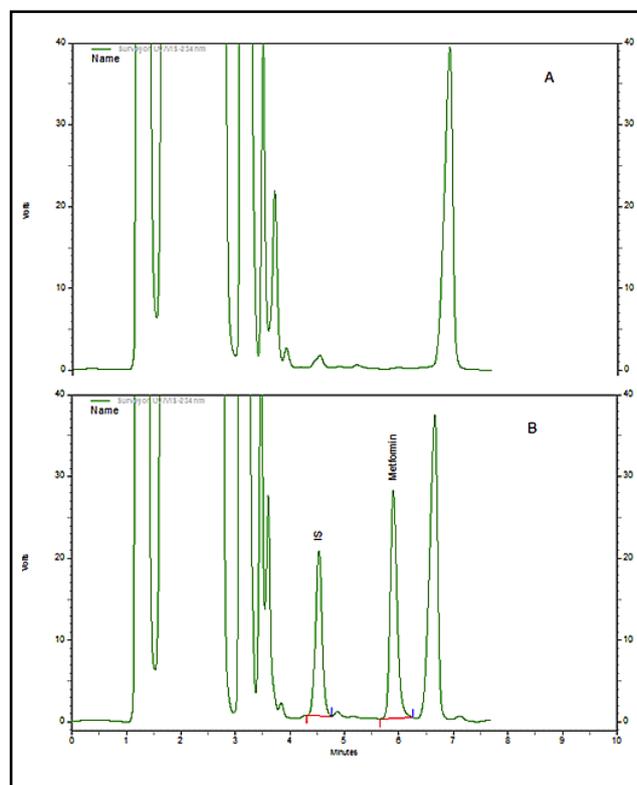


**Fig. 1.** Chromatograms of blank (A) and plasma sample containing metformin 2 250 ng/ml and internal standard 5 µg/ml (B).

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 ($R^2$) 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.

*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
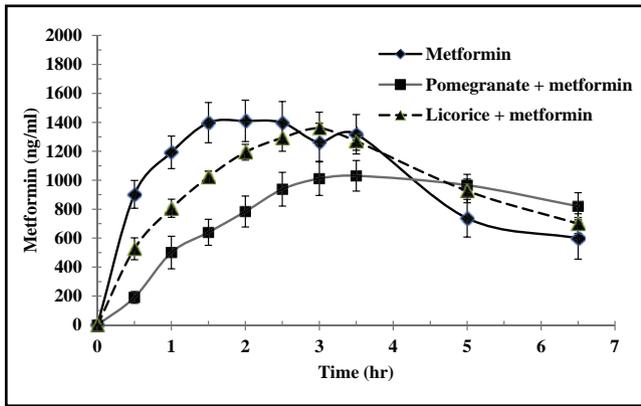


**Fig. 2.** The effect of pre-administration of pomegranate or licorice juice on plasma metformin concentration–time profile following an oral dose of metformin (20 mg/kg). Results are shown as mean ± SEM (n=9–10 rats).

**Tab. 2.** Validation results of the HPLC method used for analyzing metformin in rats' plasma.

| Parameter | Value |
|---|---|
| Linearity (R$^2$) | 0.999 |
| linearity equation | Y=0.000725X−0.002026 |
| **Accuracy (%)** | |
| intra-day accuracy (range) | (100.74–104.65%) |
| inter-day accuracy (range) | (100.99–103.75%) |
| **Precision (CV%)** | |
| intra-day precision (less than) | 4.62% |
| inter-day precision (less than) | 4.42% |
| **Stability (%)** | |
| Short term | Stable for 8 hr |
| Auto-sampler | Stable for 24 h |
| Freeze thaw | Stable for 3 cycles |
| **Recovery** (%) | 95.2±4.3 |

**Tab. 3.** Pharmacokinetic parameters for metformin in rat plasma after a single dose of metformin compared to metformin following pre-administration of pomegranate or licorice.

| Parameter | Metformin↑ | Condition | |
|---|---|---|---|
| | | Metformin + Pomegranate | Metformin + Licorice |
| Cmax (ng\ml) | 1410±138 | 1031±50* | 1359±80 |
| Tmax (hr) | 2.3±0.3 | 3.1±0.5 | 3.1±0.1* |
| AUC last (ng.hr\ml) | 6262±734 | 4762±370 | 6105±348 |

↑ values are expressed as mean ± SEM
*$p<0.05$ vs. metformin alone

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.

## ACKNOWLEDGMENT

*Competing interests*
*The authors declare that they have no competing interests.*

REFERENCES

1 Adukondalu D, Shravan Kumar Y, Vamshi Vishnu Y, *et al.* (2010). Effect of pomegranate juice pre-treatment on the transport of carbamazepine across rat intestine. Daru **18**: 254–9.

2 Banihani SA, Makahleh SM, El-Akawi Z, Al-Fashtaki RA, Khabour OF, Gharibeh MY, Saadah NA, Al-Hashimi FH, Al-Khasieb NJ (2014). Fresh pomegranate juice ameliorates insulin resistance, enhances β-cell function, and decreases fasting serum glucose in type 2 diabetic patients. Nutr Res. **34**(10): 862–867.

3 Chen MF, Shimada F, Kato H, *et al.* (1991). Effect of oral administration of glycyrrhizin on the pharmacokinetics of prednisolone. Endocrinol Jpn **38**: 167–74.

4 Dresser GK, Bailey DG, Leake BF, *et al.* (2002). Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. Clin Pharmacol Ther **71**: 11–20.

5 Farkas D, Greenblatt DJ (2008). Influence of fruit juices on drug disposition: discrepancies between in vitro and clinical studies. Expert Opin Drug Metab Toxicol **4**: 381–93.

6 Hidaka M, Okumura M, Fujita K-I, *et al.* (2005). Effects of pomegranate juice on human cytochrome p450 3A (CYP3A) and carbamazepine pharmacokinetics in rats. Drug Metab Dispos **33**: 644–8.

7 Hou Y-C, Lin S-P, Chao P-DL (2012). Liquorice reduced cyclosporine bioavailability by activating P-glycoprotein and CYP 3A. Food Chem **135**: 2307–12.

8 Howlett HC, Bailey CJ (1999). A risk-benefit assessment of metformin in type 2 diabetes mellitus. Drug Saf **20**: 489–503.

9 Jarvis S, Li C, Bogle RG (2010). Possible interaction between pomegranate juice and warfarin. Emerg Med J **27**: 74–5.

10 Mazokopakis EE, Starakis IK (2012). Recommendations for diagnosis and management of metformin-induced vitamin B12 (Cbl) deficiency. Diabetes Res Clin Pract **97**: 359–67.

11 Misaka S, Nakamura R, Uchida S, *et al.* (2011). Effect of 2 weeks' consumption of pomegranate juice on the pharmacokinetics of a single dose of midazolam: an open-label, randomized, single-center, 2-period crossover study in healthy Japanese volunteers. Clin Ther **33**: 246–52.

12 Nathan DM, Buse JB, Davidson MB, *et al.* (2009). Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care **32**: 193–203.

13 Paine MF, Criss AB, Watkins PB (2004). Two major grapefruit juice components differ in intestinal CYP3A4 inhibition kinetic and binding properties. Drug Metab Dispos **32**: 1146–53.

14 Sawada K, Yamashita Y, Zhang T, Nakagawa K, Ashida H. (2014). Glabridin induces glucose uptake via the AMP-activated protein kinase pathway in muscle cells. Mol Cell Endocrinol. **393**(1–2): 99–108.

15 Scheen AJ (1996). Clinical pharmacokinetics of metformin. Clin Pharmacokinet **30**: 359–71.

16 Setter SM, Iltz JL, Thams J, Campbell RK (2003). Metformin Hydrochloride in the Treatment of Type 2 Diabetes Mellitus : A Clinical Review with a Focus on Dual Therapy. 2991–3026.

17 Shravan Kumar Y, Adukondalu D, Bhargavi Latha A, Vamshi Vishnu Y, Ramesh G, Shiva Kumar R, Madhusudan Rao Y, Sarangapani M (2011). Effect of pomegranate pretreatment on the oral bioavailability of buspirone in male albino rabbits. Daru **19**(4): 266–269.

18 Won CS, Oberlies NH, Paine MF (2012). Mechanisms underlying food-drug interactions: inhibition of intestinal metabolism and transport. Pharmacol Ther **136**: 186–201.