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ASSESSING THE IMPACT OF PINEAPPLE AND POMEGRANATE JUICES ON IN VITRO CYP2C9-MEDIATED GLIMEPIRIDE METABOLISM

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Abstract
The primary aim of this investigation was to assess the influence of pineapple and pomegranate juices on the CYP2C9-mediated metabolism of glimepiride. The study was centered on a comparative appraisal of the inhibitory effects of pineapple and pomegranate juices using human liver microsomes in vitro. To address this inquiry, the impact of pineapple and pomegranate juices on the metabolism of another CYP2C9 substrate, glimepiride, was scrutinized through the determination of inhibition constants (IC50) and exploration of their effect on Glimepiride's Michaelis-Menten kinetics employing human liver microsomes. Subsequent to the centrifugation of incubates, samples were injected into the HPLC system, and quantification was executed by evaluating the UV peak areas at 228nm. Notably, pineapple juice emerged as a potent inhibitor of human CYP2C9 in comparison to pomegranate juice. In human liver microsomes, the average IC50 values for pineapple juice (PIJ) and pomegranate juice (POJ) concerning CYP (glimepiride hydroxylation) were identified as 1.62 ± 0.273 µl and 3.08 ± 0.388 µl, respectively. The findings obtained from in vitro experiments indicated the competitive nature of inhibition, evident from the elevated Km values (47.50 µMole) and mostly unaltered Vmax (0.492 µMole/min/mg protein) when treated with pineapple juice. A parallel observation was made for pomegranate juice, where Km increased (34.00µMole) and Vmax remained mostly unaffected (0.509µMole/min/mg protein). In essence, this study revealed modifications in the metabolism of glimepiride instigated by pineapple and pomegranate juices. Amidst the fruits subjected to evaluation, pineapple juice displayed robust inhibition of CYP2C9 activity, whereas pomegranate juice exhibited a marginal impact on the oxidative metabolism of glimepiride.

Keywords:- pineapple•pomegranate•CYP2C9 • glimepiride• human liver microsomes.

INTRODUCTION

The interplay between fruit juices and pharmaceuticals can significantly impact drug absorption and metabolism rates. CYP2C9 constitutes approximately 18% of cytochrome P450 proteins in liver microsomes and is pivotal in the metabolism of around 100 therapeutic drugs. This includes medications with narrow therapeutic windows like warfarin and phenytoin, along with routinely prescribed drugs such as acenocoumarol, tolbutamide, losartan, glipizide, and select nonsteroidal anti-inflammatory drugs. Although a paucity of reports delves into the inhibition of CYP2C9 activity by fruit extracts or juices, it remains imperative to assess the potential effects of fruit juices on CYP2C9 activity.

Pineapple (Ananas comosus, Bromeliaceae) and Pomegranate (Punica granatum, Punicaceae) have global consumption and are traditionally employed for various therapeutic purposes. Both the fruit and root of the pineapple exhibit anti-inflammatory and proteolytic attributes, while Pomegranate fruit demonstrates potential antioxidant effects, including the inhibition of low-density lipoprotein oxidation and reduction in cardiovascular diseases. These attributes drive their widespread use, thereby suggesting the possibility of interactions with drugs. However, despite these attributes, there is a dearth of literature investigating the interaction of glimepiride with pineapple and pomegranate juices.

Although existing literature demonstrates hepatic oxidative biotransformation of GLM via the CYP450 system and explores its metabolism using specific CYP2C9 variants found in Japanese subjects, in vitro studies using human liver microsomes (HLM) have not yet demonstrated oxidative biotransformation. Glimepiride, a widely used third-generation sulfonylurea in the treatment of type 2 diabetes mellitus, is efficiently absorbed after oral administration and primarily metabolized by cytochrome P450 (CYP) 2C9, with an oral bioavailability close to 100%.

The present study aimed to ascertain whether pineapple and pomegranate juices possess inhibitory potential on CYP2C9-mediated drug metabolism. Notably, no prior reports have explored drug-fruit interactions involving glimepiride. Hence, this study aimed to investigate the interaction of glimepiride, serving as a model substrate, with pineapple and pomegranate juices in the context of CYP2C9-mediated metabolism.

EXPERIMENTAL

Chemicals and Reagents

GLM was generously provided as a gift sample by Cadila Healthcare Ltd., situated in Ahmedabad, India. The procurement of Nnicotinamide Adenine Dinucleotide Phosphate, reduced tetrasodium salt (NADPH), and Magnesium chloride was accomplished through Himedia Laboratories, based in India. Ethylene diamine tetra acetic acid (EDTA), dipotassium hydrogenphosphate, and potassium dihydrogenphosphate were sourced from S.d Fine-Chem Limited, also located in India. Additionally, Methanol and Acetonitrile of HPLC grade were procured from Spectrochem India. All chemicals and reagents employed within this study adhered to the standards of analytical grade quality.

Microsomal Source

A collection of 50 human liver microsomes, comprising 0.5ml with a concentration of 20mg/ml, and encompassing a mixed gender composition, were procured from Xenotech LLC., situated in the United States. These microsomes were suspended in a medium containing 250mM sucrose, after which they were stored at an ultra-low temperature of -80°C within a deep freezer. The process of thawing the frozen microsomes involved gently immersing the vial in cold running water, followed by placement in an ice water bath until ready for use. Subsequently, a thorough vortexing was conducted to achieve a uniform suspension, and aliquots were extracted while the remaining material was preserved through deep freezing for future experiments. The specifics of the total P450 content, protein concentrations, and specific activity pertaining to each P450 isoform were consistent with the information provided by the manufacturer.

Fruit Juices

Pineapple and Pomegranate fruits were sourced from a local commercial supplier and maintained at a temperature of 4°C until their utilization. The process of obtaining fruit juice involved the extraction of the consumable part of the fruit through squeezing, followed by centrifugation and filtration to eliminate any residual matter. It is noteworthy that all the extracted samples were employed within a span of 1 hour subsequent to the squeezing and filtration procedures.

Analytical method for GLM invitro metabolism

In brief, the incubation mixtures were formulated with the following constituents: 0.1M phosphate buffer at pH 7.4, 10mM $MgCl_2$, 1mM EDTA, 10 mM NADPH, and 0.5mg/ml of microsomal protein. The concentration of glimepiride in the mixture was maintained at 10µM. Duplicate tubes, each containing the reaction mixture within the phosphate buffer and NADPH solution (10mM), were subjected to an equilibration process within a shaker incubator at 150rpm for a duration of 5 minutes at 37°C. Preliminary tests confirmed that the reduction of the substrate exhibited a linear trend over a time span of 30 minutes, using a liver microsomal protein concentration of 0.5mg/ml at 37°C. Subsequently, the commencement of the reaction was achieved by the addition of preincubated NADPH (5 minutes), and the reaction was halted through the addition of 100µl of ice-cold acetonitrile. Following this, the tubes underwent centrifugation at 10,000 rpm (4°C; 10 minutes), and aliquots of the resultant supernatant were directly introduced into an HPLC system. When deemed necessary, the volume was adjusted to 200µl with buffer.

Inhibitory effect of fruit juices on CYP2C9 Activity

Suitable volumes of pineapple juice (1, 2, 3.5, 5, and 7.5µl) and pomegranate juice (1, 3, 5, 7, and 10 µl) were carefully added to fresh tubes containing GLM. The reaction mixture, as previously described, was then introduced into the tubes and subjected to vigorous mixing for a duration of 5 seconds. Subsequently, the identical procedure as outlined earlier was carried out. The impact of pineapple and pomegranate juice on the metabolism of glimepiride was quantified by expressing the inhibitory effects as a percentage of the residual activity relative to the control condition where fruit juices were absent.

Effect of Fruit juices on MM kinetics of Glimepiride

To ascertain the apparent Michaelis-Menten constant (Km) and the maximum velocity of the reaction (Vmax), graphical plots were generated in correlation with the substrate concentration employing GraphPad Prism 5 software. To assess whether the inhibition induced by fruit juices was of a competitive or noncompetitive nature, 5µl (2.5% v/v) of pineapple juice and 7µl (3.5% v/v) of pomegranate juice were individually introduced as inhibitors. This was performed across a spectrum of GLM substrate concentrations, spanning from 2 to 100µM.

HPLC Conditions

Subsequent to the completion of the incubation process, small portions of the resulting supernatants from the centrifuged incubates were introduced into the High-Performance Liquid Chromatography (HPLC) system. The HPLC configuration encompassed essential components, including a Shimadzu LC 20 AT pump, an SPD 20A UV detector, and a rheodyne 7725 fixed injector loop (20µl) for sample injection. The separation procedure involved employing a Thermo Scientific C18 Hypersil BDS column (250 x 4.6 mm) with a particle size of 5µ, accompanied by a Phenomenex C18 guard column (4×3mm). The mobile phase composition comprised 0.1% formic acid (pH 3) and acetonitrile (55:45). The system's operational temperature was maintained at the ambient level, and the mobile phase was delivered at a controlled flow rate of 1ml/min. The quantification process was facilitated through the assessment of UV peak areas at a wavelength of 228nm.

Data analysis

In the context of this investigation, the decline of GLM within the medium subjected to incubation at a temperature of 37°C in the presence of HLM and NADPH was gauged as the proportion of the initial GLM quantity present in the medium prior to incubation. The findings were subsequently presented as a percentage, signifying the rate of turnover when necessary. The pace of substrate disappearance was computed using the formula [(C0, initial - Cs, t min) / incubation time /CYP concentration], where C0, initial symbolizes the substrate concentration at the outset (0 min), and Cs, t min represents the substrate concentration following a 30-minute incubation period with a protein concentration of 0.5 mg/ml.

The apparent kinetic attributes, specifically Km and Vmax, associated with the CYP2C9 catalyzed reaction in human liver microsomes, alongside the IC50 values denoting the inhibition of P450 activities, were evaluated using Graphpad Prism 5 software via the application of nonlinear least square regression analysis. All the outcomes were subsequently depicted as the mean arithmetic values, accompanied by their respective standard deviations (± SD).

RESULTS

Inhibitory effect of fruit juices on CYP2C9 Activity

For the assessment of the inhibitory impact of fruit juices on the activity of CYP2C9, the hydroxylation of GLM was carried out in the presence and absence of these juices using liver microsomes. As depicted in Figure 1, the inhibitory influence of pineapple and pomegranate juices on the activity of CYP2C9 was contingent upon the quantity of their respective juices incorporated into the reaction mixture. The average IC50 values determined for pineapple and pomegranate juices were established at 1.50 ± 0.23 µl (0.75% v/v) and 4.25 ± 0.53µl (2.12% v/v) respectively. At concentrations equivalent to 0.5% v/v, the inhibition percentage was calculated as 60.348%, and this value reduced to 29.42% when the concentration was elevated to 1.5% v/v for pineapple juice. Similarly, in the case of pomegranate juice, the inhibition percentage was measured at 74.067% for 0.5% v/v concentration and at 53.986% for 1.5% v/v concentration. Among the evaluated fruit juices, it was observed that pineapple juice exhibited a more pronounced inhibitory activity on CYP2C9 compared to pomegranate juice.

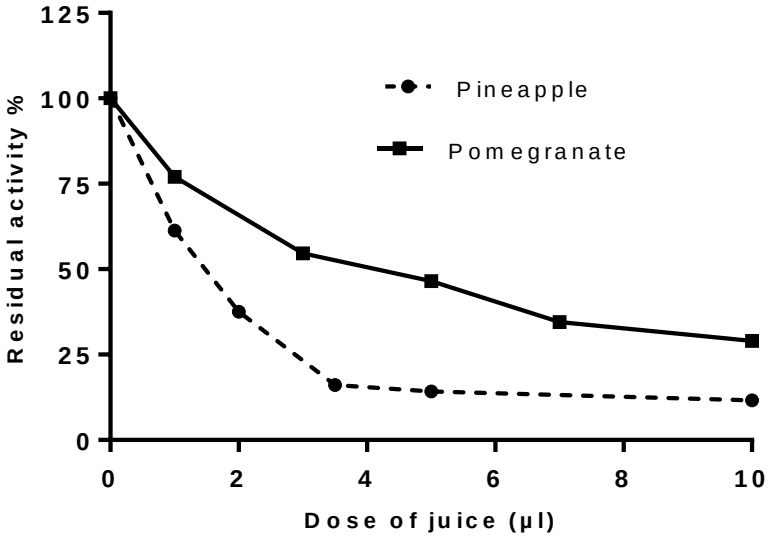

Figure 1. The inhibitory effect of PIJ and POJ juices on CYP2C9 activity.

The bar graph represents the comparative evaluation of the inhibitory potencies of pineapple and pomegranate juices on CYP2C9-mediated drug metabolism of GLM *invitro*using human liver microsomes (Fig. 2). It reflects that pineapple juice is more inhibitory in nature than pomegranate.

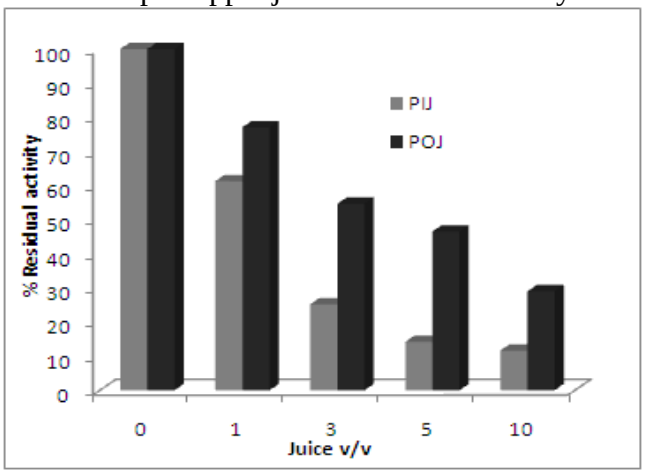

Figure 2. Bar graph representing the comparative evaluation of the inhibitory potencies of PIJ and POJ on CYP2C9-mediated drug metabolism of GLM.

Effect of Fruit juices on MM kinetics of Glimepiride

As depicted in Figure 3, the results obtained from the in vitro experiments indicated a competitive form of inhibition, signified by the elevation of Km values (47.50 µMole) while maintaining the Vmax (0.492 µMole/min/mg protein) relatively unaffected in the case of pineapple juice. In a parallel manner, the application of pomegranate juice led to an increase in Km (34.00µMole) and minimal alteration in Vmax (0.509µMole/min/mg protein). A concise compilation of the kinetic parameters associated with the metabolism of glimepiride is provided in Table 1.

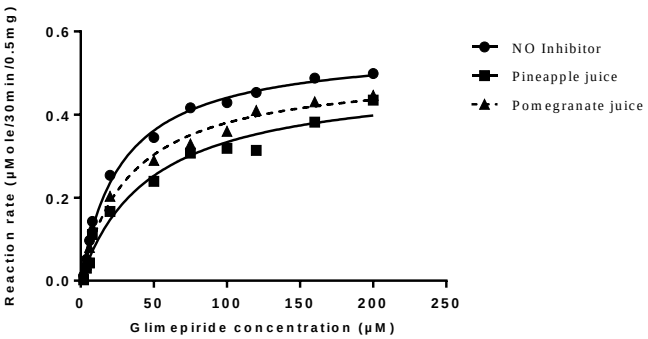

Figure 3. Effect of PIJ and POJ on MM kinetics of GLM.

Table 1. Summary of the kinetic parameters of GLM metabolism.

#	Kinetic parameters	Observed values
1	Km	28.9 ± 2.77 µMole
2	Vmax	0.559 ± 0.015 µMole/min/mg protein
3	IC50 (Pineapple juice)	1.62 ± 0.273 µl
4	IC50 (Pomegranate juice)	3.08 ± 0.388 µl
5	Km (in presence of Pineapple juice)	47.50 ± 2.3 µMole
6	Vmax (in presence of Pineapple juice)	0.492 ± 0.038µMole/min/mg protein
7	Km (in presence of Pomegranate juice)	34.00 ± 3.15µMole
8	Vmax (in presence of Pomegranate juice)	0.509 ± 0.021µMole/min/mg protein

DISCUSSION

Limited reports are available regarding the potential interactions between food and drugs facilitated by fruit juices. In this investigation, we delved into the impact of pineapple and pomegranate juices

on the in vitro activity of CYP2C9, shedding light on the plausible interactions between these juices and CYP2C9 substrates in human systems.

Inhibition constants (IC50) derived from in vitro analyses hold the potential to offer quantitative insights into the prediction of pharmacokinetic food-drug interactions. Based on a comprehensive evaluation of results encompassing Km, Vmax, and IC50 values, it became evident that pineapple juice exercises a discernible competitive inhibitory influence compared to pomegranate juice on the metabolism of GLM.

The relatively subdued inhibitory potential exhibited by pomegranate juice toward the in vitro metabolism of GLM raises prospects for favorable effects in individuals grappling with type 2 diabetes. The potential merits of pomegranate juice warrant further exploration through clinical investigations to substantiate its distinctive antidiabetic attributes. Nevertheless, before incorporating it into regular dietary routines, a more comprehensive understanding of its potential health contributions is imperative.

Among the various fruits scrutinized, pineapple emerged as a robust inhibitor of CYP2C9 activity (as displayed in Table 1). The addition of 10 µl (5% v/v) of pineapple juice resulted in near-complete inhibition.

Addressing diabetes mellitus through dietary strategies holds promise, with pomegranate juice being a potential participant. The restrained inhibitory influence of pomegranate juice on the in vitro metabolism of GLM suggests favorable implications for individuals dealing with type 2 diabetes. Pomegranate juice assumes the role of a healthful fruit juice, inviting further clinical investigations to strengthen its unique antidiabetic attributes. Literature underscores the potency of pomegranate juice, seed oils, and flower extracts in managing insulin resistance and glucose metabolism [8-17].

Evidence suggests that the consumption of pomegranate juice is linked to improved insulin efficiency, reduced insulin resistance, enhanced lipid control, and better blood sugar management [11-12]. Notably, pomegranate juice sugars, distinctively bonded with antioxidants, seem to offer protective effects against diabetes and atherosclerosis, a surprising discovery [13]. This counterintuitive result stems from the fact that sugars in pomegranate juice, unlike those in other juices, adopt a form that is not detrimental but rather health-supportive.

While our in vitro findings champion the use of pomegranate juice as a potential asset for individuals with diabetes, comprehensive investigations are imperative to decipher its full scope in contributing to human health. This warrants due diligence before endorsing its routine consumption.

CONCLUSION

This investigation has illuminated shifts in the metabolism of GLM due to the influence of PIJ and POJ. Notably, the introduction of a mere 10 µl (5% v/v) of pineapple juice led to a nearly complete inhibition of GLM metabolism. Within the spectrum of fruits explored, PIJ emerged as a potent suppressor of CYP2C9 activity, while POJ displayed a relatively minor role in modulating the oxidative metabolism of GLM. It is crucial to exercise caution while extrapolating inhibitor effects from in vitro settings to in vivo scenarios. Furthermore, it should be acknowledged that the impact of fruit juices on drug pharmacokinetics in vitro might not seamlessly align with their effects in human subjects. Consequently, a deeper understanding of these dynamics necessitates further exploration through human studies.

The in vitro assessment of interactions between drugs and fruit juices suggests a greater inhibitory tendency of pineapple juice compared to pomegranate juice. This insight underscores the significance of vigilant monitoring in diabetic patients when considering the concomitant consumption of these juices.

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COMPETING INTERESTS

The author(s) declare(s) no conflict of interest.

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