ORGINAL ARTICLE

Pharmacokinetic Drug Food Interaction Study of Nateglinide and Pomegranate Fruit Juice

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Abstract

Objective: Pomegranate juice is inhibitor of CYP450 enzyme system such as CYP2C9 and CYP3A4. The objective of the present study was to determine the consequence of pomegranate fruit juice on the pharmacokinetics of oral hypoglycemic drug nateglinide in rats.

Materials and Methods: This is a laboratory study to investigate drug-food interaction effects of Punica granatum fruit juice (3 ml) and nateglinide (in doses 10 and 20 mg/kg body weight, p.o.) in live animals where the effects of food drug interaction on pharmacokinetics parameters such as Cmax and AUC in vehicle and nateglinide and pomegranate juice and nateglinide treated rats was undertaken. Four groups of Wistar albino rats comprising of (n=5) animals in each group were taken and treated with vehicle and nateglinide (in doses 10 and 20 mg/kg body weight, p.o.) pomegranate juice (3ml, p.o.) and nateglinide (in doses 10 and 20 mg/kg body weight, p.o.).

Results: The rats which were administered with pomegranate juice + nateglinide (in doses 10 and 20 mg/kg body weight, p.o.) showed raised C_{max} to 2.85 fold and 2.21-fold respectively and an increase in AUC was found to be 1.34 fold and 1.47 fold respectively, when vehicle + nateglinide 10mg/kg and vehicle + 20mg/kg drug treated groups were compared. The results were compared at *P*-value< 0.01.

Conclusion: A significant drug interaction was observed when nateglinide and pomegranate juice was administered indicating caution must be exercised when such food and drug is co-administered as the chance of more hypoglycemia may occur due to this potential drug-food interaction.

Keywords: Food–Drug interaction, Pomegranate, Nateglinide

Introduction

Food-drug interactions are defined as modification of drug disposition or action of a drug or dietary component or a deficiency in dietary status as a result of the adding of a drug (1). A study has detailed that when grapefruit juice and felodipine or nifedipine, which are calcium channel antagonists were co-administered has resulted

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in a great enhancement in the oral bioavailability of these drugs and an augmentation of their pharmacological actions. (2). One study suggested that pomegranate juice may influence the removal of medicines that are substrate of the CYP3A4 enzymes that should be put in its precise medical deliberation (3). Also one of the studies has showed constituents of Punica granatum fruit juice reduced the CYP3A-mediated biological degradation of carbamazepine. In addition, Punica granatum fruit juice produces effects on the drug disposition in rats (4). Among the CYP450 enzyme system the CYP3A4 is the principal isoform of CYP3A in mature humans. (5).

Punica granatum L (family Punicaceae), also called as pomegranate fruit, is generally eaten fruit around the world, and it has been in use for a broad range of remedial reason in traditional medicine (6). It has been reported that Punica granatum fruit possesses definite species of flavonoids and anthocyanins in its seed oil and juice and it demonstrates strong antioxidant activity, resulting in useful health effects such as reduction in the low-density lipoprotein oxidation and decline in the cardiovascular diseases (7-9). Increased consumption of pomegranate fruit may lead to a higher likelihood of Punica granatum fruit juice-drug interaction. Hence, it becomes imperative to evaluate the resultant effects between Punica granatum fruit juice and CYP3A-mediated metabolism of drugs as such interactions were not reported.

The study has undertaken whether Punica granatum fruit juice could inhibit CYP3A4 mediated drug metabolism of nateglinide which is a substrate of CYP3A4 for in vivo study.

Materials and Methods

The present study was undertaken to investigate the drug-food combined effects among Punica granatum fruit juice and nateglinide in live animals. For the experimentation the fruit Punica granatum (Pomegranates) were procured from fruit market of Aurangabad district of Maharashtra stae. Pomegranates were amassed at 5°C till they were used. Pomegranates were cut in to parts, the peel was detached and the seeds grinded in mixer to obtain freshly prepared juice which was administered to rats (n=5) by taking five animals in each group the statistical significance can be achieved. The gift sample of active pharmaceutical ingredient (API) of nateglinide was obtained from Wockhardt Research Center, Aurangabad.

Suspensions of nateglinide 10mg/kg and 20mg/kg were prepared in 0.5% CMC to obtained strength 4mg/kg and 8mg/kg dose volume 2.5 ml/kg was selected. Rats of Wistar albino strain having weight of 200-220 g were accommodated in standard confined mesh boxes at temperature (22± 2 °C) with humidity (40± 5%), under a 12 h light/dark cycle and were fed the regular pellet diet and drinking water as required, were used for study.

Animals were at random separated in four groups (n= 05 in each group).The first group received vehicle [0.5%, (CMC) 0.5 ml p.o.] for 10 days and on 11^{th} day nateglinide (10mg/kg, p.o.) was administered; the second group received vehicle [0.5%, (CMC) 0.5 ml p.o.] for 10 days and on 11^{th} day nateglinide (20mg/kg, p.o.) was administered, the third group received pomegranate juice 3ml (p.o.) for 10 days, and on 11^{th} day animal single dose of nateglinide (10mg/kg, p.o.), the fourth group received pomegranate juice 3ml (p.o.) for 10 days, and on 11^{th} day animal single dose of nateglinide (20mg/kg, p. o.). The animals were grouped in to four set 1, set 2, set 3, and set 4 to assist alternate bleeding. In this study overnight fasted rats were dosed with 3ml of pomegranate juice administered orally 1 hour before the administration of drug on 11^{th} day. Five animal were alternately bleed at each time point (0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12 Hrs) 0.5 ml blood was withdrawn per time point by retro–orbital plexus under carbogen (70:30 CO_2 and O_2) anesthesia. By the process of centrifugation at 10, 000 rpm the blood samples were subjected for 300 seconds at 4°C and the obtained part of blood i.e. plasma were

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transferred individually in respective Eppendorff tubes for each time point labeled and immediately stored at - 60°C until analysis by LC–MS/MS.

Samples were processed by protein precipitation method to extract the active drug from the study sample. The stock solution for nateglinide (1mg/kg) was prepared in methanol and these stocks were externally spiked in to same blank plasma (W. Rat) by performing the serial dilutions. 10µg/ml to 0.039µg/ml for nateglinide 50µl of linearity standard and test sample were precipitated with 400µl acetonitrile and vortexed for 2 min. then 50µl of Milli Q water containing internal standard (Repaglinide 2 mcg/ml) was transferred and further mixed with the help of vortex mixer for 2 minutes further all the test sample were subjected to centrifugation process at 4°C for 3 minutes at 10,000 revolution per minute and submitted for LC–MS/MS analysis.

Plasma nateglinide concentration was calculated by LC-MS with the help of Absciex Q Trap API 4500 LCMS/MS system, the LCMS/MS system was kept in the chemical ionization mode under normal atmospheric pressure, repaglinide was used as IS. The ion transition monitoring were m/z 318.2–166.2 for nateglinide, m/z 453.4–230.2 for repaglinide. The monitored transitions correspond to the result ions of the $[M+H]^+$ ions. LC-MS system consist of Zorbax XDPC 18 column (50× 2.1 mm 5µ with Guard column) 10 mm ammonium formate adjusted to pH 4.2 with formic acid used as buffer and this buffer and acetonitrile used for solvent in proportion of 20:80 v/v, flow rate was 0.3 ml/minute, injection volume was 0.2µl for nateglinide. Column temperature was 35°C and run time was 2.6 min. the concentration of linearity for nateglinide was 10µg/ml to 0.039 µg/ml. The pharmacokinetic parameters such as Cmax and AUC were selected as parameters to be measured. Results are expressed as Mean ± SD (Standard deviation) analyzed by student unpaired T-test. Variation among means were measured statistically significant if *P*-value< 0.01. The complete records were analyzed with the help of statistics program system SPSS-21.

Ethical considerations

The experiment protocol for this study was approved with an approval number CPCSEA/IAEC/P'col-38/2014-15/91 by an Institutional Animal Ethical Committee (IAEC), Wockhardt, Ltd under the guidance of CPCSEA, Ministry of Forest and Climate Change, Government of India.

Results

The Cmax values of nateglinide 10 mg/kg and 20 mg/kg were found to be significantly higher in the groups 3 and 4 treated with pomegranate juice as compared to the CMC vehicle treated groups 1 and 2 (*P*-value< 0.001) respectively (Figure 1, 2). The plasma concentrations in terms of mean AUCs of nateglinide with pomegranate juice were found to be appreciably increased in rats (grouping three and four) as judge against to rats treated with CMC vehicle treated groups one and two (*P*-value< 0.01) respectively (Figure 3, 4). The pharmacokinetic parameters are summarized in (Table 1).

Discussion

Since after the finding that grapefruit juice hampers cytochrome P-450 3A4 (CYP3A4) in the small intestinal wall, a lot of works have been completed to additionally identify interactions with grapefruit juice and more in recent times, with other fruit juices. These studies have lead to an enhanced consideration of how medicines are biologically degradation and carried inside the biological system and how usually occurring stuff can influence these processes. Studies laboratory information confirms that Punica granatum fruit juice always hampers CYP2C9 and CYP3A4 intestinal enzymes. Hence Punica granatum fruit juice may enhance the bioavailability of medicines that are biologically degraded by these enzymes. But, study reports in man discovers that Punica

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Table 1. Effect of pomegranate juice on pharmacokinetic parameters of nateglinide in rats

Groups	Treatment	Pharmacokinetic Parameters	
		C_{max} (µg/ml)	AUC (µg. hr/ml)
Group 1	Distilled water Nateglinide (10 mg/kg, p.o.)	0.40 (±0.03)	0.85 (±0.02)
Group 2	Distilled water + Nateglinide (20 mg/kg, p.o.)	1.16 (±0.07)	1.14 (±0.06)
Group 3	Pomegranate Juice (3ml)+ Nateglinide (10 mg/kg, p.o.)	1.04 (±0.09)$^{\#}$	1.91 (±0.08)$^{\$}$
Group 4	Pomegranate Juice (3ml) + Nateglinide (20 mg/kg, p.o.)	2.30 (±0.09)$^{\#}$	2.8214 (±0.09)$^{\$}$

Result are expressed as Mean ± SD (n=5) analyzed by Student unpaired T-test $^{\$}$ *P*-value < 0.01 and $^{\#}$ *P*-value < 0.001 when judged against with group 1 & 2. SD= Standard Deviation, C_{max}= Concentration maximum, AUC= Area under the curve.

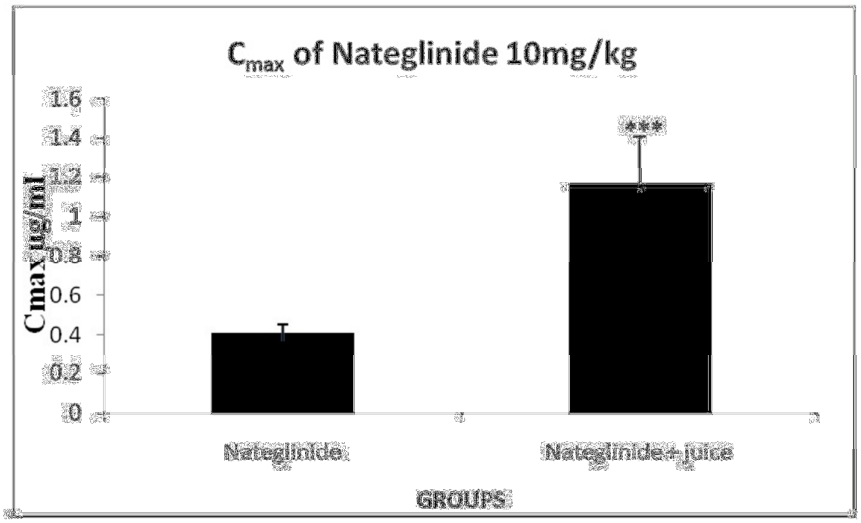

Figure 1. Comparison of C_{max} of Vehicle + Nateglinide with Nateglinide 10mg/kg + Pomegranate Juice
***** Indicates a significant variation among group 1 and group 3 (*P*-value < 0.001).**

granatum fruit juice does not enhance exposure to either CYP2C9 or CYP3A4 substrates (10).

Nateglinide is classified under the meglitinide oral hypoglycemics. It varys from repaglinide in being biologically degraded by CYP2C9 (70%) and CYP3A4 (30%). The current study deals with identification and evaluation of potential interaction caused by Punica granatum when taken along with oral hypoglycemic drug. According to data obtained from our study, it was found that Punica granatum juice when administered with oral hypoglycemic drug which is a CYP2C9 and CYP3A4 substrate caused to change in plasma concentration of the drug.

According to data obtained by LC-MS/MS analysis, when nateglinide10mg/kg and 20mg/kg was administered with pomegranate juice, it was found that Cmax was increased by 2.85-fold and 2.21-fold respectively when compared with vehicle + nateglinide 10mg/kg and vehicle + nateglinide 20mg/kg drug treated group.

The AUC of nateglinide 10mg/kg and 20mg/kg with pomegranate juice, was found to be raised by 1.34-times and 1.47-times correspondingly when judged against with vehicle + nateglinide 10 mg/kg and vehicle + nateglinide 20mg/kg drug treated group.

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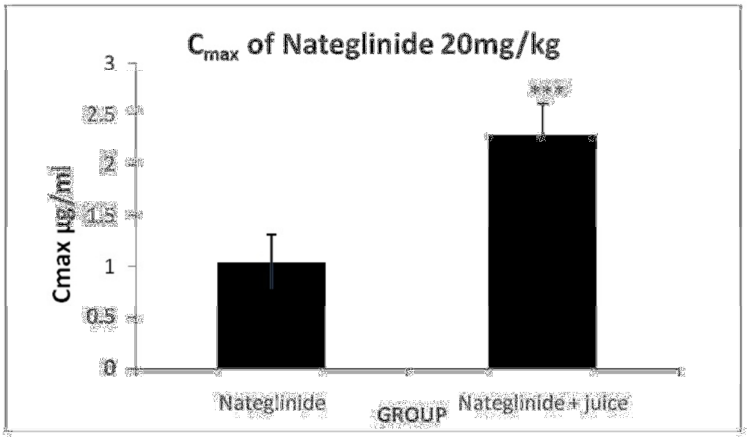

Figure 2. Comparison of C_{max} of Vehicle + Nateglinide with Nateglinide 20mg/kg + Pomegranate Juice
***** Indicates a significant difference among group 2 and group 4 (*P*-value < 0.001).**

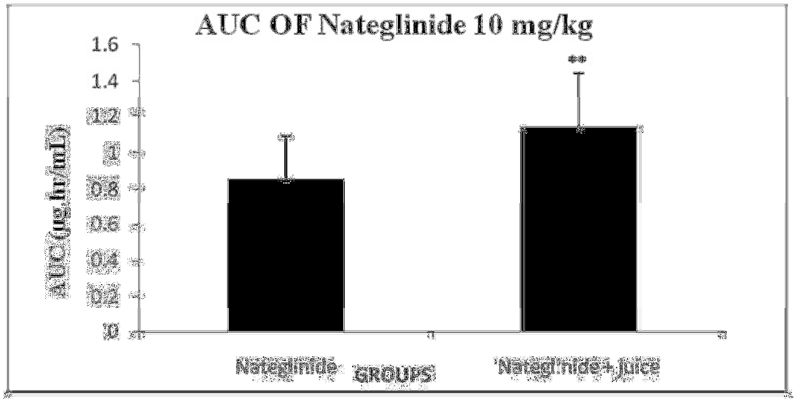

Figure 3. Comparison of AUC of Vehicle + Nateglinide with Nateglinide 10mg/kg + Pomegranate Juice
**** Indicates a significant variation among group 1 and group 3 (*P*-value < 0.01).**

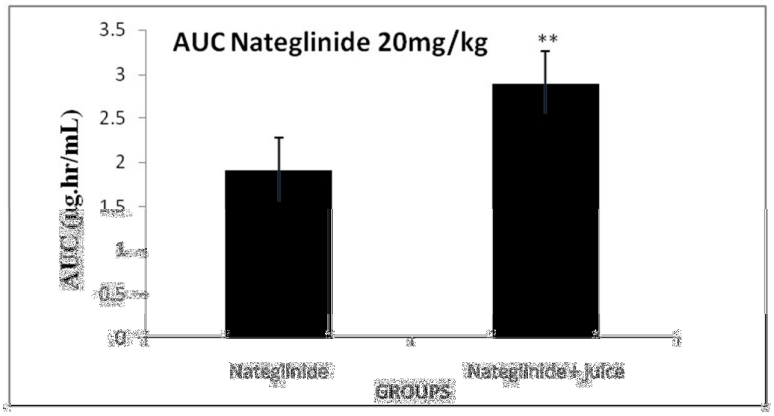

Figure 4. Comparison of AUC of Vehicle + Nateglinide with Nateglinide 20mg/kg + Pomegranate Juice
**** Indicates a significant difference among group 2 and group 4 (*P*-value < 0.01).**

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In the year 1998, different researchers demonstrated that grapefruit juice, in common, is a potent inhibitor of CYP3A4, which can influence the biological degradation of a range of medicines, raising their blood concentrations. In few cases, this can lead to a lethal interaction with medicines like astemizole or terfenadine (11). The action of grapefruit juice with regards to medicines absorption was initially discovered in 1989. The primary available information on grapefruit drug interactions was in 1991 in the Lancet under the title of interactions of citrus juices with felodipine and nifedipine and was the primary information on food-drug interaction in humans. Nevertheless, the action became gained publicity following being accountable for a number of deaths due to overdose on medication. The action of grapefruit remains up to 3-7 days, with the peak effects within 24 hours of use. In adding to grapefruit, other fruits have alike effects. Noni (M. citrifolia), for instance, is a nutritional supplement in general eaten as a juice and also hinder CYP3A4, Punica granatum fruit juice has this action as well (12).

Masashi Nagata et al. studied whether Punica granatum fruit juice could hinder CYP2C9 activity. The capacity of Punica granatum fruit juice to hinder the diclofenac 4-hydroxylase activity of human CYP2C9 was observed through human liver microsomes. Punica granatum fruit juice has been revealed to be a powerful inhibitor of human CYP2C9. The adding up of twenty five µl (5% v/v) of Punica granatum fruit juice produced end result in nearly absolute hindrance of human CYP2C9 action. In adding up, they examined the actions of Punica granatum fruit juice on the drug disposition of tolbutamide (substrate for CYP2C9) in animal rats. In relation to the normal animal group, the area under the concentration-time curve was approximately 1.2-times larger when Punica granatum fruit juice (3 ml) was administered oral route 1hour prior to the intake of the tolbutamide (20 mg/kg). The elimination half-life of tolbutamide was not changed by Punica granatum fruit juice intake. These outcome suggest intake inhibits the intestinal degradation of tolbutamide without inhibiting the hepatic degradation in laboratory animals. Therefore, they revealed that Punica granatum fruit juice repressed human CYP2C9 action and also augmented unchanged tolbutamide blood levels in animals (13).

The increase in the plasma concentration of drug could be due to presence and involvement of membrane ATPase carrier protein, p-glycoprotein, present in higher concentration in enterocyte in small intestine. As it was accounted that administration of certain food like citrus fruit juices for instance grape fruit juice and sevile orange juice can change the blood concentration of dextromethorphan, where it caused an raise in blood levels and absorption of dextromethorphan by affecting first pass degradation and absorption through intestinal gut-wall (14). One study has collated and evaluated in the animals and humans shows facts of the ability of Punica granatum fruit juice to be an executor in drug-drug interactions intervened by CYP3A4 and CYP2C9 with the help of available invitro data (15).

Strengths

It's a prospective study indicating a potential food drug interaction between pomegranate juice and oral hypoglycemic drug nateglinide. In order to prevent the profound fall in blood sugar level and further worsening of diabetic patient's condition when this drug and pomegranate juice is administered this study will be helpful.

Limitations

Although the study design selected is enough but the small sample size is the limitation in this study.

Conclusions

In the present report we have shown that Punica granatum fruit juice by inhibiting the

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CYP2C and CYP3A-mediated metabolism of nateglinide could produce an increase in the plasma concentration of nateglinide which may cause pronounced hypoglycaemia which could be undesirable. Such interactions must be studied in humans so that an advice to the patient can be given in order to avoid further complications of the sufferer's disease.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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