International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 1465-1469
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Enhancement of Hypoglycemic Activity of Sitagliptin In Presence of Naringenin: An Herb-Drug Interaction Study in Diabetic Rats

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Received: 10 Jan 2019 / Accepted: 9 Mar 2019 / Published online: 1 Apr 2019
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Abstract
Sitagliptin (SG) is a DPP-4 inhibitor class of oral anti-diabetic drug and commonly used by most of the diabetic patients. SG was mainly metabolized by CYP enzymes. The present study was conducted to know the changes in different pharmacokinetic (pk) and pharmacodynamic (pd) parameters of drug in presence of naringenin. Streptozocin was used for induction of diabetes in rats. The rats were divided in different groups and administered with suitable doses of sitagliptin and naringenin. In normal and diabetic rats pk parameters like C_{max}, AUC, MRT, t½ were slightly increased, whereas volume of distribution, clearance was decreased due to the inhibition of CYP3A4 enzyme. The Combination of sitagliptin with naringenin improves the hypoglycemic activity and serum insulin levels in diabetic rats. Finally, this herb-drug combination led to the changes in different pk/pd and should be used cautiously.

Keywords
Sitagliptin (SG), Naringenin (NR), CYP enzymes, Herb-Drug interaction, biochemical parameters.

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INTRODUCTION
Diabetic patients are using different herbal preparations along with allopathic medicines prescribed by medical practitioners. Most of the Indian plant products and food ingredients contain anti-diabetic properties [1]. Co-administration of drug-herb may lead to interactions in terms of pharmacokinetics (pk) and pharmacodynamics (pd) of drug. The changes in pk & pd parameters effect the therapeutic activity of drug.

Sitagliptin (SG) is a dipeptidyl peptidase-4 (DPP-4) inhibitor class of anti-hyperglycemic drug used to control the blood glucose levels in diabetic patients and it was mainly metabolized by CYP enzymes (CYP3A4 and CYP2C8)[2]. Chemically Naringenin (NR) is a flavonoid and found in citrus species, grapefruit, tomatoes, cocoa. Previous reports stated that naringenin have anti-diabetic properties and inhibitory effect on CYP3A4 enzymes [3].

MATERIALS AND METHODS
Sitagliptin (SG) was obtained as gift sample from Novartis, Hyderabad and Naringenin (NR) was purchased from yucca enterprises, Mumbai. Streptozocin (STZ) was supplied frSom sisco laboratories, Methanol HPLC grade supplied from Merck chemicals, Mumbai. All the chemicals used for the study are of analytical grade.

HPLC analysis of Sitagliptin

20:80 (v/v) mixtures of methanol and distilled water (pH adjusted to 3.0 with orthophosphoric acid) was used as mobile phase and delivered at a flow rate of 1 ml/min. The mobile phase was degassed by sonicator and filtered through 0.22 µm membrane filter. At 266nm of wavelength the detection was carried out and total run time was adjusted to 10min [4].

Diabetes induction in rats

Induction of diabetes in rats Streptozocin (STZ) at a dose of 55mg/kg was used for induction of diabetes in rats. The required quantity of STZ was dissolved in citrate buffer (pH 4.5) and given through i.p route of administration. Blood samples were collected from rats after 72hrs by retro-orbital puncture and glucose levels were analyzed in serum sample. Rats with blood glucose level >250 mg/dL were considered as diabetic and were used for the study [5].

Pharmacokinetic study in normal and diabetic rats

Overnight fasting, rats were divided into normal and diabetic groups (n = 6). Both groups of rats were administered with sitagliptin (25mg/kg) and pretreated with naringenin (30 mg/kg) for 7 days and on the 8th day with sitagliptin (25 mg/kg) followed by naringenin. Through retro orbital plexus blood samples were collected by using heparinized capillary tubes, and immediately same volume of normal saline was replaced intra peritoneally [6]. The blood was collected at time intervals of 0.5, 1, 2, 4, 8, and 24 hrs in every group. Serum was separated after centrifugation at 3000 rpm for 20 min and the samples were stored at – 20°C until analysis [7]. The pharmacokinetic parameters like C_{max}, T_{max}, AUC_{total}, $t_{1/2}$, MRT, Cl and V_d were calculated.

Pharmacodynamic study in diabetic rats

After induction of diabetes by using STZ, rats were fasted overnight and divided into 4 groups (n = 6). The rats of group I (diabetic control, normal saline), group II (Sitagliptin, 25mg/kg), group III (Naringenin, 30mg/kg) and group IV (Sitagliptin, 25mg/kg+ Naringenin, 30mg/kg) were treated orally. Blood samples were drawn at 0 (Initial fasting blood sample), 0.5, 1, 2, 4, 6, 12 and 24h from the retro-orbital plexus of the rats. The samples were analyzed for blood glucose levels using glucose oxidase-peroxidase method and percentage reduction in blood glucose concentrations were determined [8,9].

Statistical analysis: The Pharmacokinetic parameters were calculated by using Kinetica™ software (version 4.4.1). All values of pharmacokinetic and pharmacodynamic studies were expressed as Mean±SD. The data were statistically evaluated using one-way analysis of variance (ANOVA). Results were statistically significant when $p \leq 0.05$.

RESULTS & DISCUSSION:

Pharmacokinetics of sitagliptin in diabetic rats:

In diabetic pretreated rats, compared with the group (given sitagliptin alone), the co-administration of naringenin significantly ($p<0.05$) increases C_{max} (1.25 and 1.38 times), AUC_{total} (1.13 and 1.34 times), $t_{1/2}$(1.04 and 1.12 times), MRT (1.04 and 1.12 times), whereas the clearance (1.2 and 1.7 times) and volume of distribution (1.39 and 2.2 times) of sitagliptin was decreased. The T_{max} was not altered significantly in both normal and diabetic rats. Naringenin inhibits the CYP3A4 enzyme involved in the metabolism of the drug and thereby availability of drug was increases. Combination of herb-drug, leads to increase in pharmacokinetic parameters like C_{max}, AUC_{total} & decrease in volume of distribution and clearance due to the changes in metabolism of sitagliptin by inhibitory action of Naringenin on CYP enzymes [10].

Table 1: Mean pharmacokinetic parameters of sitagliptin STZ-induced diabetic rats.

PK parameter	Normal rats		Diabetic rats	
	SG	SG+NR	SG	SG+NR
C_{max} (µg/ml)	3.14±0.28	3.85±1.37	4.26±0.53	5.35±1.68*
T_{max} (h)	2	2	2	2
AUC_{total} (h.µg/ml)	104.6±5.27	146.2±8.36*	125.42±4.58	194.6±7.25 *
$t_{½}$ (h)	8.15±1.03	8.53±1.18	8.47±1.24	9.56±2.19 *
MRT (h)	9.31±1.65	10.6±1.13	10.25±1.37	11.6±2.6 *
CL (ml/h/kg)	96.12±10.3	73.81±9*	74.16±12.8	42.81±9.57*
V_d (ml/kg)	627.6±18.4	450.27±23.5*	485.2±15.4*	214.8±21.3*

All values are expressed as mean ± SD (n=6)

Significant at *p<0.05 considered as when compared to control groups.

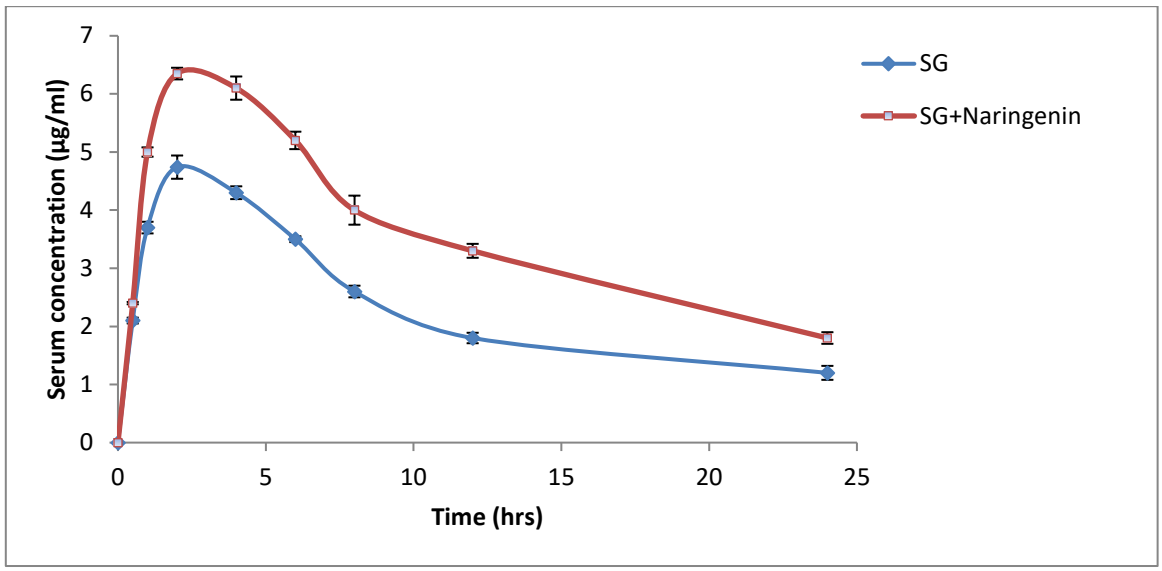

Figure.1: Mean serum concentration of sitagliptin in different groups of diabetic rats.

Pharmacodynamic study in different groups of diabetic rats

Mean serum glucose level and percentage glucose reduction in diabetic rats were calculated. The data indicates that there is a maximum reduction of serum glucose level observed in group treated with combination of sitagliptin and naringenin (Group- IV, 47.1%), when compared with standard (Group- II, sitagliptin, 39.47%), Naringenin (Group- III, 24.8%) alone treated groups. Significant decrease ($p \leq 0.001$) in serum glucose levels at different time intervals were observed upon co-administration of sitagliptin with naringenin [11].

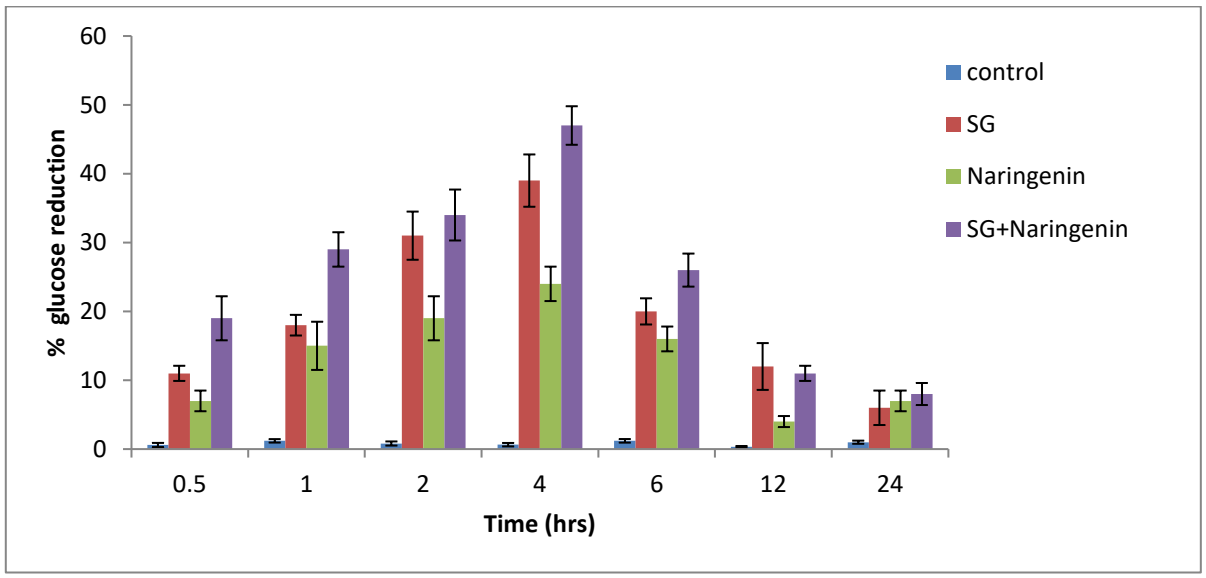

Figure.2: Comparison of percentage glucose reduction in different groups of diabetic rats.

After 28 days of study, serum insulin (biochemical parameter) levels were maximum (42.1%) in NR-sitagliptin group and was well comparable to that of the standard sitagliptin (29.4%) and NR (20.4%) alone treated groups. Naringenin with sitagliptin combination have exhibited more serum insulin levels and there by blood glucose was decreased [12,13].

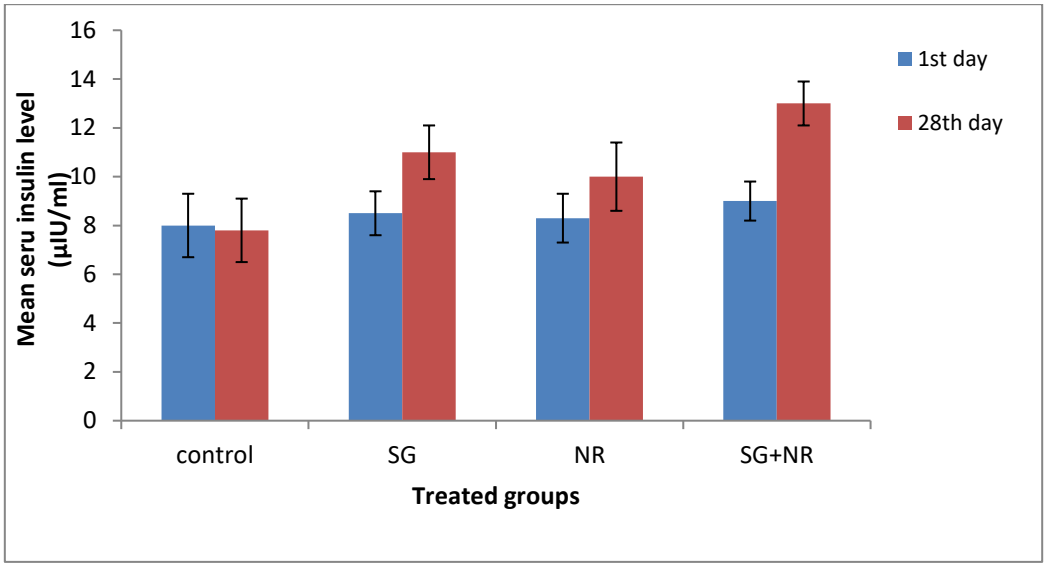

Fig.3: Effect of Naringenin, sitagliptin & combination of both on serum insulin levels in diabetic rats

CONCLUSION:

Naringenin (NR) inhibits the CYP3A4 enzyme activity and there by sitagliptin metabolism was decreases and increases the availability of drug. The blood glucose levels were reduced, and serum insulin was improved in presence of naringenin with sitagliptin. The interaction was beneficial in terms of reduction in blood glucose level in diabetic rats. Due to this, drug doses may require special attention if it is administered with NR containing preparations.

ACKNOWLEDGEMENTS:

The authors are thankful to Novartis, Hyderabad, India for providing the gift sample of sitagliptin.

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