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Effect of Nicardipine on Pharmacokinetics and Pharmacodynamics Sitagliptine in Normal and Diabetic rats

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ABSTRACT: The objective was to study the drug-drug interaction between Nicardipine and Sitagliptine in normal and alloxan induced diabetic rats. The study was designed in four phases. In the first phase, Nicardipine was administered (10mg/kg; p.o) followed by the administration of Sitagliptine (300mg/kg; p.o) and the blood glucose levels in normoglycemic rats was studied (pre-treatment on the hypoglycemic activity studied). Simultaneously the influence of Nicardipine treatment for seven consecutive days on blood glucose levels was also studied in normoglycemic rats. In the second phase of the study alloxan-induced diabetic rats were used to find out the influence of Nicardipine pre-treatment on sitagliptine induced hypoglycemic effect in pathophysiological condition. Blood samples were collected from retro-orbital plexus at regular intervals of 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 24.0 h after drug treatment. All the blood samples were analyzed for plasma glucose by glucose oxidase peroxidase method (GOD/POD). In the third phase sitagliptine was estimated by reverse phase HPLC method in Normal rats and and in the fourth phase sitagliptine was estimated in diabetic rats using reverse phase HPLC method. The therapeutic dose of Nicardipine potentiates the hypoglycemic activity of Sitagliptine both in normoglycemic and diabetic rats respectively. The results indicate that the dose of oral hypoglycemic agents needs to be adjusted if co-administered with Nicardipine.

KEYWORDS: Drug-drug interaction, Nicardipine, Hypoglycemic, Sitagliptine.

I. INTRODUCTION

Drug interaction: An interaction is said to occur when the effects of one drug are changed by the presence of another drug, herbal medicine. The outcome can be harmful if the interaction causes an increase in the toxicity of the drug.

Patients often receive several drugs at the same time because diseases such as cancer, diabetes and AIDS, demand the need for combination therapy, which works better than can be achieved with any one of the drugs alone. An interaction may occur between them by either altered pharmacokinetics or pharmacodynamics of one drug by another.

For a drug with a narrow therapeutic window, only a small change in response may precipitate a clinically significant interaction, whereas for a drug with a wide margin of safety, large changes in its pharmacokinetics will have no clinical consequence, but some interactions are intentional. Hence to safeguard the delicate health of diabetic patients it is appreciable to have accurate information on glycemic regulation achieved by commonly prescribed sitagliptine when co-administered with Nicardipine. In this investigation, it was designed to evaluate the effect of Nicardipine on glycemic regulation achieved by Sitagliptine in normal and alloxan induced rats.

II. MATERIALS

Sitagliptine & Nicardipine were procured from matrix pharmaceuticals, Hyderabad, India, in 2011. The glucose estimating kits, R.K.Diagnostics, Karimnagar, India, all other chemicals used were of HPLC grade. Wistar rats procured from mahaveera Enterprises (Hyderabad, Telangana, India), weighing between 180 to 250 GMS. The experiments were planned after the approval of Institutional Animal Ethical Committee. (Regd No.576/02/bc/CPCSEA).

III. METHODS

ESTIMATION OF SITAGLIPTINE BY A SENSITIVE RP-HPLC METHOD:

A waters 2487 HPLC system used in the study.
Mobile phase: 10mM sodium dihydrogen phosphate (PH-4.6) and acetonitrile in the proportion of 50:50v/v. The buffer solution filtered through 0.45 µm membrane filter. Flow rate: 1ml/min, Λmax: 267nm, Run time: 10min

International Journal of Pharmaceutical Research and Applications
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Preparation of calibration curve of sitagliptine for invivo samples:

Preparation of stock solutions: A stock solution representing 1mg/ml of sitagliptine was prepared in methanol and this solution was stored at -20°c until use. Standard solutions were prepared prior to use from the stock solution by sequential dilution with methanol to yield concentrations of 25, 50, 75, 100, 200, 300, 400, 500 and 1000ng/ml of sitagliptine. The internal standard (Pioglitazone) stock solution was prepared representing 1mg/ml of this solution was stored at -20° until use.

Extraction procedure:

In a 2ml micro centrifuge tube, 100µl of internal standard solution was taken. The plasma was precipitated by the addition of 100µl of methanol and then the tubes were vortexed for 3min. and centrifuged at 7000rpm for 10min. the supernatant was transferred to a clean, similarly labelled tube from this 20 µl was injected into the HPLC.

Construction of calibration curve:

Construction of calibration curve was constructed using peak height ratios of drug to the internal standard vs. concentration. The slope of the plot was determined by the method of linear regression analysis was used to calculate the sitagliptine concentration in the unknown sample. A linear calibration curve in the range of 10mcg to 100mcg was established (r^2 =0.997). Retention times of internal standard sitagliptine were 5.3 and 7.1 respectively.

ESTIMATION OF BLOOD GLUCOSE LEVELS BY GOD-POD METHOD

Glucose is oxidised by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalysed reaction p-hydroxy benzoate and 4-amino antipyrine react with hydrogen peroxide to form red coloured quinine complex. Absorbance data measured at 510nm directly proportional to glucose concentrations.

Preparation of working reagent

Dissolve the contents of glucose reagent with glucose diluent, swirl the mixture gently to dissolve the contents, do not shake vigorously. After collection of blood sample into a micro centrifugation tube containing anticoagulant and it was centrifuged at 3000rpm for 15min then the plasma glucose was estimated.

Experimental induction of diabetes: Experimental diabetes in rats was induced by the administration of alloxan monohydrate in two doses of 100mg and 50mg/kg body weight, intraperitoneally for 2 consecutive days. After 72hr, sample was collected from rats by orbital puncture of all surviving animals and the serum was analyzed for glucose levels. Rats with blood glucose levels of 200mg/dl and above were considered as diabetic and selected for the study.

Pharmacodynamic and Pharmacokinetic evaluation in diabetic rats:

Group I (control) ------ 0.2 ml of 0.5% sodium CMC solution; p.o

Group II----------Sitagliptine was administered for 8 days (300mg/kg; p.o)

Group III---------- Nicardipine was administered (10mg/kg; p.o) followed by the administration of Sitagliptine (300mg/kg; p.o)

Group IV----------Pretreated with Nicardipine (10mg/kg; p.o) for 7 days, on the 8th day Nicardipine (10mg/kg) and Sitagliptine (300mg/kg; p.o) were administered.

In the acute study and as well as in chronic study, blood samples were collected from orbital sinuses at time intervals between 0, 0.5, 1, 2, 3, 4 , 6, 24 hrs in normal and treated groups in both pharmacokinetic and pharmacodynamic methods.

Blood glucose levels were determined using GOD-POD method in pharmacodynamic interaction. Serum was separated by centrifugation using Bio-fuge and stored in vials at -20ºC until further analysis by pharmacokinetic method.

Sitagliptine was estimated by reverse phase HPLC method. Above mentioned pharmacokinetic and pharmacodynamic interaction study was also done in the Normal rats.

IV. RESULTS :

Sitagliptine concentrations were estimated by a sensitive RP-HPLC METHOD and the typical chromatogram corresponding the blank plasma and plasma samples were obtained.

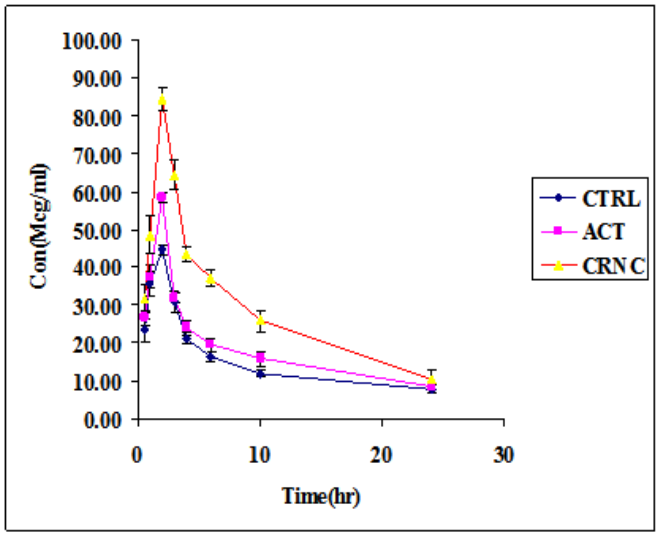
PHARMACOKINETIC DATA IN NORMAL RATS:

Mean plasma Sitagliptine concentration (µg/ml) before and after oral administration of Nicardipine in Normal rats (n=6):

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 216-222 www.ijprajournal.com ISSN: 2249-7781

Time (hrs)	Sitagliptine	Sita+Nic (acute)	Sita+Nic(chronic)
0	0	0	0
0.5	23.30 ± 3.00	26.71 ± 2.07	31.57 ± 3.69
1	35.78 ± 3.38	37.59 ± 3.049	48.5 ± 4.96
2	44.50 ± 1.36*	58.32 ± 1.05*	84.36 ± 2.88*
3	30.29 ± 2.95	32.12 ± 1.44	64.35 ± 3.91
4	23.17 ± 1.58	24.01 ± 1.98	43.50 ± 1.86
6	17.34 ± 2.01	19.60 ± 1.97	37.02 ± 2.24
10	12.02 ± 0.89	15.8 ± 1.96	25.92 ± 2.74
24	6.82 ± 1.53	8.41 ± 0.78	10.39 ± 2.57

Comparision of Mean ± S.D plasma concentration time profile of sitagliptine (300mg/kg) before and after oral administration of Nicardipine in Normal rats (n=6)



The sitagliptine concentrations in plasma were significantly altered by Nicardipine on acute and chronic treatment

Mean plasma Sitagliptine concentration (µg/ml) before and after oral administration of Nicardipine in Diabetic rats (n=6):

Time (hrs)	Sitagliptine	Sita+Nic (acute)	Sita+Nic (chronic)
0	0	0	0
0.5	22.30 ± 2.97	21.51 ± 1.23	26.85 ± 2.60
1	32.78 ± 3.37	32.91 ± 1.56	38.57 ± 7.43
2	41.49 ± 1.38*	54.98 ± 2.45*	58.04 ± 2.55*
3	32.98 ± 2.94	43.68 ± 3.47	46.38 ± 2.99
4	21.16 ± 1.53	32.49 ± 5.78	37.04 ± 1.87
6	16.34 ± 1.88	20.42 ± 2.50	19.52 ± 2.65
10	11.99 ± 0.88	14.17 ± 1.71	16.64 ± 1.77
24	7.82 ± 1.13	10.20 ± 2.01	15.12 ± 1.79

The data was expressed as mean ± standard error of the mean. The significance was determined by Paired Student's t test. Data were computed for statistical analysis using Graph Pad Instat and prism software. The results were considered statistically significant if $P<0.05$.

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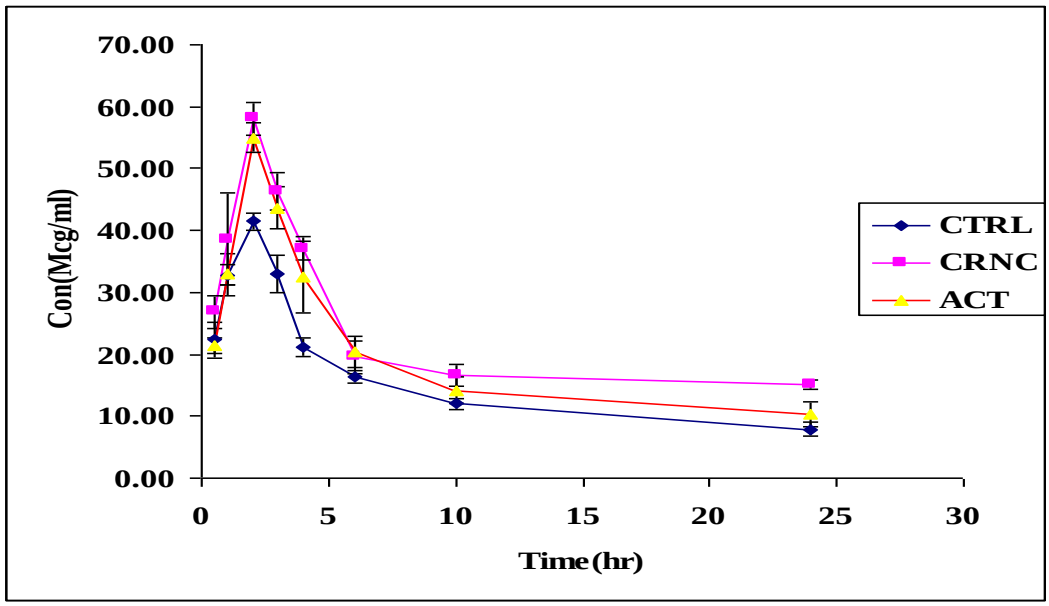
Comparision of Mean ± S.D plasma concentration time profile of sitagliptine (300mg/kg) before and after oral administration of Nicardipine in Normal rats (n=6):



The sitagliptine concentrations in plasma were significantly altered by Nicardipine on acute and chronic treatment.

Comparision of Pharmacokinetic parameters of sitagliptine (300mg/kg) following pretreatment with Nicardipine (10mg/kg) by oral administration in diabetic rats (n=6).

Parameter	Sitagliptine	Sita+Nic(acute)	Sita+Nic(chronic)
AUC(ng/ml/hr)	351.33 ± 15.24	420.42 ± 14.21 (1.1 9 times)	662.60 ± 22.41 (1.88 times)
AUC(tot)	538.99 ± 22.63	604.22±22.30 (1.1 2 times)	818.48 ± 34.03 (1.51 times)
Cmax(ng/ml)	43.10 ± 1.87	58.33 ± 1.06 (1.35 times)	84.70 ± 3.00 (1.96 times)
Tmax(hr)	2 ± 0.0 0	2.00 ±0.0 0	2.00 ± 0.00
t1/2 (hr)	17.20 ± 3.58	15.03 ± 2.22 (0.87 times)	10.00 ± 2.44 (0.58 times)
CL(ml/hr)	0.18 ± 0.03	0.17 ±0.01 (0.94 times)	0.12 ± 0.01 (0.66 times)
MRT(hrs)	22.61± 4.67	20.09 ± 2.81 (0.88 times)	14.21 ± 3.07 (0.62 times)

Comparision of Pharmacokinetic parameters of sitagliptine (300mg/kg) following pretreatment with Nicardipine (10mg/kg) by oral administration in diabetic rats (n=6)

Parameter	Sitagliptine	Sita+Nic(acute)	Sita+Nic(chronic)
AUC(ng/ml/hr)	357.13 ± 16.45	447.41 ± 30.58 (1.25 times)	423.83 ± 12.56 (1.18 times)
AUC total	564.17 ± 20.34	652.81 ± 41.22 (1.1 5 times)	640.31 ± 33.24 (1.13 times)

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 216-222 www.ijprajournal.com ISSN: 2249-7781

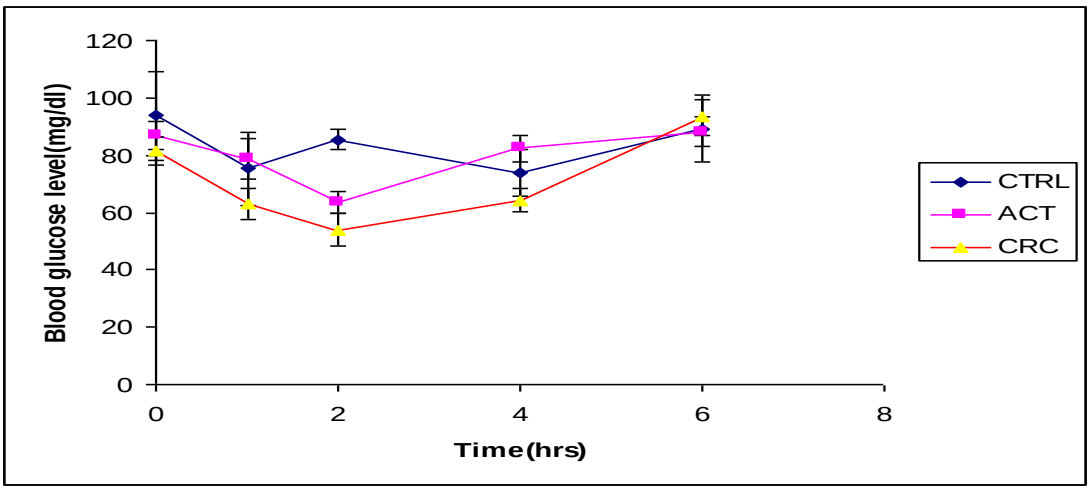
Cmax(ng/ml)	41.50 ± 1.26	55.01 ± 2.23 (1.32 times)	48.05 ± 2.55 (1.1 5 times)
Tmax(hr)	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
t1/2 (hr)	18.26 ± 3.69	19.30 ± 5.70 (1.0 5 times)	17.50 ± 5.72 (0.9 5 times)
CL(ml/hr)	0.18 ± 0.03	0.14 ± 0.02 (0.07 times)	0.15 ± 0.02 (0.83 times)
MRT(hrs)	23.97 ± 4.90	25.76 ± 7.49 (1.07 times)	23.54 ± 6.30 (0.98 times)

PHARMACODYNAMIC DATA

Mean ±SD reduction of blood glucose profile of sitagliptine (300mg/kg) following pretreatment with Nicardipine (10mg/kg) by oral administration in Normal rats:

Time (hrs)	**Sita(std) (mean ± S.D)**	**Sita+Nic(acute) (mean ± S.D)**	**Sita+Nic(chronic) (mean ± S.D)**
0	93.67 ± 10.74	86.83 ± 4.70	81.5 ± 4.78
1	75.4 ± 11.06	78.66 ± 7.25	63 ± 5.29
2	85.46 ± 26.78	63.5 ± 4.03*	54 ± 5.47*
4	73.78 ± 16.73	82.33 ± 4.63	64.33 ± 4.06
6	89.23 ± 11.82	84.21 ± 11.09	75.24 ± 14.5

Comparision of Mean ± S.D plasma concentration time profile of sitagliptine (300mg/kg) following pretreatment with Nicardipine by oral administration in Normal rats.



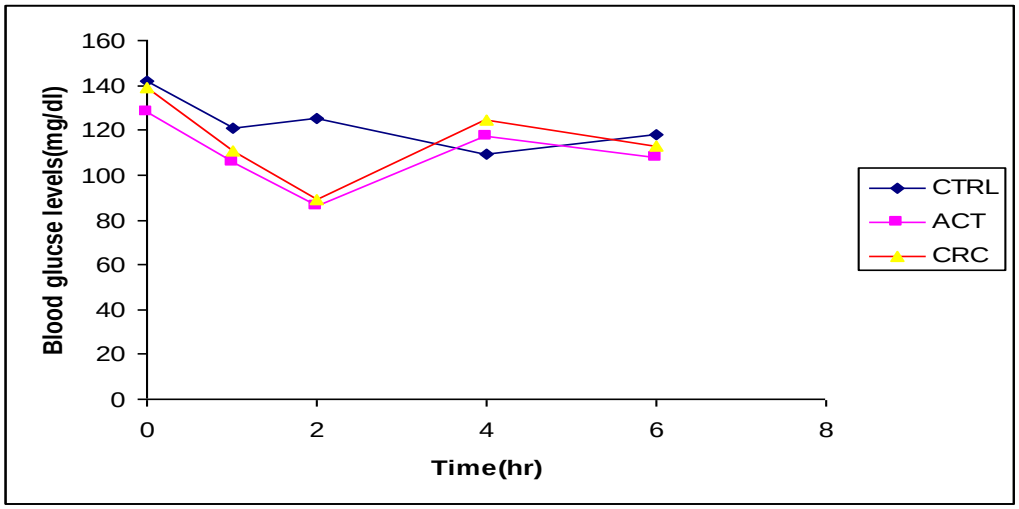
Mean±SD reduction of blood glucose days profile of sitagliptine (300mg/kg) following oral administration in diabetic rats:

Time (hrs)	**Sita(std) (mean ± S.D)**	**Sita+Nic(acute) (mean ± S.D)**	**Sita+Nic(chronic) (mean ± S.D)**
0	142 ± 30.92	128.33 ± 14.90	138.67 ± 11.08

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 216-222 www.ijprajournal.com ISSN: 2249-7781

1	121 ± 28.33	117.17 ± 9.54	124.67 ± 8.64
2	125 ± 26.73	105.50 ± 9.41*	110.67 ± 8.70
4	109 ± 28.05	85.83 ± 18.25	89.17 ± 14.90
6	118 ± 24.09	102..21 ± 11.57	93.24 ± 7.90

Comparision of Mean ± S.D plasma concentration time profile of sitagliptine (300mg/kg) following pretreatment with Nicardipine by oral administration in Diabetic rats.



V. CONCLUSION

Sitagliptine is a DPP-4 inhibitor that blocks the breakdown of the incretin hormones GIP and GLP-1; it reduces fasting plasma glucose and decreases postprandial hyperglycaemia and is metabolized by CYP isoenzymes CYP3A4, CYP2C8, CYP2C9.

Nicardipine is a calcium channel blocker (dihydropyridine), acts on smooth muscles and reduces the peripheral vascular resistance and mainly used in hypertension and angina.

It is reported that Nicardipine is mainly metabolized by liver microsomal enzymes CYP2C8, CYP3A4 and minorly CYP1A2, CYP2C9,and CYP2D6. It is also reported that it is an inhibitor of CYP3A4 and CYP2C8.

"The possible mechanism behind this type of interaction in healthy & diabetic rats on single and multiple dosages may involve inhibition of CYP3A4 & CYP2C8 the enzymes, responsible to metabolise sitagliptine in liver & GIT".

The present study results suggest that, initial treatment (single dose studies in normal rats) with combination of Sitagliptine and Nicardipine enhanced the bioavailability of Sitagliptine. In multiple dose studies Nicardipine increased the antidiabetic activity of Sitagliptine by increasing the bioavailability of Sitagliptine and these results were found to be statistically significant ($P < 0.05$).

Well designed controlled studies in human beings are needed to confirm the interaction, So the initial investigations reveal that combination of Sitagliptine and Nicardipine should be used with caution in a clinical situation, especially in patients with diabetes and hypertension.

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