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To study the Influence of Nifedipine on the Pharmacokinetics of Sitagliptin in Rats

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ABSTRACT:
Diabetes mellitus (DM) is a condition of increased blood glucose levels in the body. Sitagliptin, a novel therapeutic agent for the treatment of type 2 DM is a selective inhibitor of enzyme dipeptidyl peptidase 4 which metabolises the incretin hormones in control of blood glucose levels. Nifedipine, a calcium channel blocker used for the treating patients with severe hypertension. Clinically Sitagliptin is given as an oral antidiabetic drug to treat DM. Nifedipine may be co-prescribed along with Sitagliptin to treat hypertension respectively. As such, no information is available regarding the interaction taking place between Sitagliptin / Nifedipine. Hence the present work has been undertaken to find out the interaction taking place between the above said drugs in rodent model, since such studies cannot be performed in humans.

KEYWORDS:sitagliptin, nifedipine, animal models, incretins, drug interactions, hyperglycemia, hypoglycemia.

I. INTRODUCTION:

Diabetes mellitus (DM) is a syndrome characterized by hyperglycemia, altered metabolism of carbohydrates, lipids, and proteins, and increases risk of cardiovascular diseases. Diabetes mellitus is one such disorder, which requires careful management of its therapy with respect to blood glucose level since hyperglycaemia and hypoglycaemia are unwanted.In the present day, the number of patients suffering from disorders like diabetes and its associated co-morbidities like atherosclerosis,dyslipidemia, and other cardiac disorders is increasing worldwide. This study is planned to establish the safety of the drug combinations in animal models with respect to blood glucose levels, serum insulin levels, and serum Sitagliptin concentration and find out the mechanism responsible for the interactions if any.

II. BACKGROUND:

Diabetes mellitus (DM) is a syndrome characterized by hyperglycemia, altered metabolism of carbohydrates, lipids, proteins and increases the risk of cardiovascular diseases. Among diabetes, approximately 95% of patients have type-2 DM, whereas about 5% have type-1[1]. The increasing prevalence of type-2 diabetes itself may confer 75-90% of the excess risk of enhancing micro vascular complications like diabetic retinopathy, nephropathy, neuropathy and macro vascular complications like coronary diseases, hypertension, cardiac myopathy, cerebrovascular diseases and peripheral vascular diseases[2].Sitagliptin is a new class of oral drug and novel therapeutic agent for treatment of type 2 diabetes[8]. Sitagliptin is a selective, competitive and fully reversible DPP-4 inhibitor that was discovered through the optimization of a class of β-aminoacid-derived DPP4 inhibitors[10]. It lowers DPP4 activity in a sustained manner following oncedaily administration, preserves the circulating levels of intact GIP and GLP1 following meals in both acute and chronic studies and reduces blood glucose levels without significantincreases in hypoglycaemia[11].It act mainly by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4) which metabolises the naturally occurring endogenous incretin hormones, glucagon –like peptide-1(GLP-1) and glucose- dependent insulinotropic polypeptide (GIP) which are responsible for the release of insulin[12], GLP-1 and GIP resulting in enhanced glucose-dependent insulin secretion from the pancreas & decreased hepatic glucose production to maintain glucose homeostasis[9]. GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells by intracellular signalling pathways. Glucose induces insulin secretion by a signalling pathway involving glucose uptake via the glucose transporter GLUT2. Glucose metabolism increases the (Adenosine-triphosphate/ Adenosine-diphosphate) ATP/ADP ratio, which induces closure of the (Potassium) K+/ATP-dependent channel, depolarization of the plasma

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membrane, opening of the voltage-dependent (Calcium) Ca++ channel (VDCC) and closure of the voltage-dependent K+ channel (Kv). Calcium entry into beta cells induces insulin granule exocytosis. On binding to their specific receptors, GIP / GLP-1 have similar signalling pathways that activate adenylate cyclase and increase intracellular cyclic adenosine mono phosphate (cAMP) levels. The effects of cAMPare then mediated by two ubiquitouslyexpressed intracellular cAMP receptors: the classic protein kinase A (PKA) /cAMP-dependent protein kinase; and the recently discovered exchange protein directly activated by cAMP, (Epac) /cAMP-regulated guanine nucleotide exchange factor. Protein kinase phosphorylates different targets (GLUT2, K+/ATP channel, VDCC, Kv). Epac (PKA) stimulates the release of calcium from the endoplasmic reticulum by ryanodine (RYR) receptors and stimulates insulin granule exocytosis [10].

Nifedipine which crosses the blood brain barrier also block L-type calcium channel particularly in dopaminergic neurons in the CNS. Theseblockade appeared to provide a protective effect, with a significant decrease in the risk of developing Parkinson's disease[11]. Since insulin release is the major mechanism in Sitagliptin activity and its release depends on the activity of K^+ ATP channels and intracellular signaling pathway involved. It is likely that the drugs affecting the above mechanisms may precipitatedrug interactions at the receptor level leading to decreased effect of K^+ ATP channel openers and decreased / increased effect of Nifedipine. The information on the safety of such classes of drugs in combination with Sitagliptin is scanty. Sinceconcomitant administration of Sitagliptin with Nifedipine is followed in diabetics associated with hypertension. Hence, there might be possibility for drug-drug interaction with enhance / decrease Sitagliptin activity, which is unwanted. The safety of the above drug combinations with respect to blood glucose is not known and needs to be established by preclinical and clinical studies.

For pre-clinical evaluation of drug interactions, rodents like mice, rats and nonrodents like rabbits are used as experimental animal models. The pharmacodynamic / pharmacokinetic parameters at preclinical levels can be conveniently studied in them. The above animals can be easily maintained in laboratory conditions and small volumes of blood can be withdrawn easily at regular time intervals. Hence in the present study albino wistar rats (rodent model) were selected for study of pharmacokinetic drug interactions and to identify the mechanisms of drug interactions. Such interactions are likely to occur in humans also, if it occurs in the above said animal species. Such studies in animal model may help in predicting the mechanism of interaction and the results can be extrapolated to humans to provide safe combinations in clinical situation.

III. METHODS:

Studies were conducted in normal and alloxan induced diabetic rats with oral doses of 9 mg / kg B.W of Sitagliptin, 18 mg /kg B.W of Nifedipine and their combinations with adequate washout periods in between the treatments. Blood samples were collected at regular time intervals in rats through retro orbital puncture. All the blood samples were analyzed for blood glucose by GOD / POD method in pharmacodynamic studies and for pharmacokinetic studies. The serum Sitagliptin concentrations were estimated by UV Spectrophotometry. The pharmacokinetic parameters such as AUC, AUMC, Vd, K_a, K_e, C max, T max of serum Sitagliptin calculated using Ramkin Software and serum insulin by chemiluminescence assay.

ANALYTICAL METHODS USED IN THE STUDY:

- Estimation of blood Glucose by GOD / POD methods.
- Blood serum Sitagliptin concentration estimation by UV-Spectrophotometry
- Serum insulin by chemiluminescence assay.

BLOOD GLUCOSE ESTIMATION:- GLUCOSE OXIDASE–PEROXIDASE (GOD/POD) METHOD

Glucose kit based on Trinder's (1969) methods in which glucose oxidase (GOD) and peroxidase (POD) enzymes were used along with the chromogen 4-aminoantipyrine and phenol. This method is one step, simple and rapid.

PRINCIPLE: Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalysed reaction the oxygen liberated is accepted by the chromogen system to give a red coloredquinineimine compound. The red colour so developed is measured at 505 nm and is directly proportional to glucose concentration.

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$$\text{Glucose} + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{P-hydroxybenzoate} \xrightarrow{\text{Peroxidase}} \text{Quinone dye}$$

REAGENTS:

Reagent-1	Glucose reagent (9 vials)	Glucose oxidase, Peroxidase, 4-aminoantipyrine, Buffer, Stabilizers
Reagent-2	Glucose diluents (1*450 ml)	Diluent, Phenol preservative
Reagent-3	Glucose standard (100 mg/dl) (1*3 ml)	Dextrose, Benzoic acid

Store Reagent-1 at 2-8^0 C and Reagent-2 at room temperature

WORKING REAGENT PREPARATION: The contents of 1 vial of Reagent-1 were transferred quantitatively to clean black coloured plastic containers provided in the kit. The bottle was reconstituted with 50 ml of glucose diluents (Reagent-2).

STORAGE OF WORKING REAGENT: Stable from 12 months from the date of reconstitution when stored at 2-8^0 C.

SPECIMEN COLLECTION: Serum was separated within 30 min from the collection of blood sample.

Equipments:-The basic assay parameters are
Mode : End point
Wavelength: 505 nm [490-550 nm]
Temperature : 37^o C or R T
Optical path length : 1 cm
Blanking: Reagent blank
Incubation: 10 min at 37^o C
Sample Volume : 10 µl
Working reagent volume : 1 ml
Concentation of standard : 100 mg/dl
Linearity: up to 500 mg/dl
Stability of color : 1 hour
MaximumAbsorptionlimit : 2
Units: mg/dl

PROCEDURE:

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Serum or plasma	-	-	10µl
Glucose standard	-	10µl	-
Working reagent	1ml	1ml	1ml

They were mixed well and incubated at 37^o C for 10 minutes and absorbance was read at 505 nm against a reagent blank.

CALCULATION: Glucose conc. = $\frac{\textbf{AbsorbanceofTest (T)}}{\textbf{AbsorbanceofStandard(S)}} \times \textbf{100}$

NOTE: Unused working glucose reagent was refrigerated immediately.

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ESTIMATIONOFSERUM SITAGLIPTIN CONCENTRATION BY UV-SPECTROPHOTOMETRY[7]:

PROCEDURE FOR ESTIMATION OF SITAGLIPTIN IN SERUM:

Absorbance of 1ml of serum were measured at 265nm against blank. The obtained absorbance values were subjected to calculate the amount of Sitagliptin present in the serum by calculating molar absorptivity of each serum sample.

$C = A/EB$,

C=Concentration, A=Absorbance, E=Molar absorptivity, B=path length.

PROCEDURE FOR ESTIMATION OF NIFEDIPINE AND SITAGLIPTIN IN SERUM BY SIMULTANEOUS METHOD:

Absorbance of collected serum were measured at 267nm and 238 nm against serum blank. The obtained absorbance values were substituted in the following equations to get the concentration of each drug.

For Sitagliptin

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2}$$

A1=Absorbanceof Sitagliptin at 267nm. A_2=Absorbance of Sitagliptin at 238nm. ax_1=molar absorptivity of A_1, ax_2=molar absorptivity of A_2

For Nifedipine

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

A1=Absorbanceof Nifedipine at 267nm. A_2=Absorbance of Nifedipine at 238nm. ax_1=molar absorptivity of A_1, ax_2=molar absorptivity of A_2

ESTIMATIONOFSERUMINSULINLEVELSBYCHEMILUMINESCENCEASSAY:

INSTRUMENTDESCRIPTION: The ADVIA Centaur CP Insulin assay.

Intended Use: Forin-vitro, the diagnosticuse in the determination of insulin in serum using the ADVIA Centaur CP Insulin Chemiluminescenceassay Systems. This assay can be used to aid in the diagnosis of diabetes mellitus and hypoglycaemia.

TABLE-1: STUDIES ON THE INFLUENCE OF CALCIUM CHANNEL BLOCKERS (NIFEDIPINE) / AMANTADINE ON THE PHARMACOKINETICS OF SITAGLIPTIN IN NORMAL HEALTHY RATS

Pharmacokinetic Significance	**Phase III**	**Normal Rats** Group I- Normal Group II Test	**Stage I**	All groups administered with vehicle control & blood samples were collected at different time intervals for blood glucose estimation.
			Stage II	Three test groups for interaction study were administered with the therapeutic dose of Sitagliptin (TD) (9 mg/kg body weight) and blood samples were collected for estimating blood glucose levels.
			Stage III	Test animals were treated with interacting drugs, Group-II: Nifedipine (18 mg/kg B.W) and blood samples were collected for estimating blood glucose levels.
			Stage IV	Test animals were treated with combination. Group-II: Nifedipine followed by Sitagliptin after 30 min.and blood samples were collected for estimating blood glucose levels.

Note: i) A washout period of 6 days were maintained in between all the stages.

ii) The blood samples were withdrawn by retro orbital puncture at 0, 1, 2, 3, 4, 6, 8,10 and 12 hours time intervals from the overnight fasted animals for analysis.

iii) Diabetes was induced in rats by the administration of Alloxan monohydrate in two

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doses, i.e. 100 mg and 50 mg/kg body wt (Intra peritoneal)i.pfor two consecutive days.

iv) Blood glucose levels were estimated using glucose oxidase-peroxidase method in Semi Auto Analyser.

v) Serum Sitagliptin levels were estimated using UV-Spectrophotometry.

vi) Pharmacokinetic parameters to be analysed were Area under the curve (AUC), Area under the first moment curve (AUMC), Plasma half-life ($t_{1/2}$), Clearance (CL), Volume of distribution of steady state (Vdss), Volume of distribution Vdarea, Peak plasma concentration (C_{max}), and Time to maximal serum concentration (T_{max}).

vii) Serum insulin levels were analysed by Chemiluminescence assay.

MATERIALS AND PROCUREMENTS: The drugs and chemicals used in the study were obtained as gift samples and purchased from various sources as detailed below.

INSTRUMENT FOR GLUCOSE ESTIMATION: SEMI AUTO ANALYZER

Glucose Kits: Glucose kits used for the estimation of serum glucose were purchased from Span Diagnostic Ltd. India.

Sitagliptin: Gifted fromActis Pharmaceuticals Pvt. Ltd., Hyderabad.

Nifedipine: Gifted fromKumar organic Pvt. Ltd., Bangalore.

Alloxan Monohydrate: Purchased from Rolex chemical industries, Mumbai

Animals:

Normal albino wistar rats (Rattus norvegicus) of either sex weighing between 175-250 g were procured from registered breeders (Sri Raghavendra Enterprises, Bangalore). The animals were housed under standard conditions of temperature (25 ± 2° C) and relative humidity (30-70%) with a 12:12 hr light dark cycle. The animals were fed with standard pellet diet and water ad libitum. The protocol was approved by the Institutional Animal Ethical Committee (IAEC) (Ref. No. IAEC/SKVCP/PGCOL/11-12/04) of Sri K.V College of Pharmacy, Karnataka (117/1999/CPCSEA) was undertaken for conducting drug interaction studies.

Alloxan induced diabetes: Alloxan is the next most commonly used chemical for induction of diabetes mellitus. It is a well- known diabetogenic agent widely used to induce Type 2 diabetes in animals[14].

Dose Determination:

The usual human therapeutic dose (TD) of Sitagliptin is 100 mg, Nifedipine 20 mg. The doses of all interacting drugs were calculated by extrapolating the human therapeutic dose to animals (rats) based on body surface area and were found to be 9 mg/kg B.W of Sitagliptin, 18 mg/kg B.W Nifedipine[13].

STUDIES ON THE INFLUENCE OF CALCIUM CHANNEL BLOCKER NIFEDIPINE ON THE PHARMACOKINETICS OF SITAGLIPTIN IN NORMAL HEALTHY RATS

The experiment was conducted in following stages in overnight fasted animals to study the pharmacokinetic interactions in normal healthy rats.

Table-2: Two groups of rats for pharmacokinetic interaction studies in healthy rats.

GROUPS	TREATMENT	No. of ANIMALS
Group-I	Normal Control	6
Group-II	Nifedipine (18 mg/Kg) + Sitagliptin (9 mg/Kg) (p.o)	6
	Total	12

Route of administration: p.o

Dose: Sitagliptin (9 mg/kg), Nifedipine (18 mg/kg).

Stage-I: All groups of rats which were fasted for overnight were administered with vehicle control and blood samples were collected at different time intervals and were estimated for blood glucose levels, serum Sitagliptin, and serum insulin levels.

Stage-II: After a washout period of six days three test groups of rats (Group II) which were fasted for overnight were administered with the TD of Sitagliptin (9 mg/kg body weight) and blood samples were collected at different time intervals and were estimated for blood glucose levels, serum Sitagliptin and serum insulin levels.

Stage-III: After a washout period of six days, the same groups of animals rats which were fasted for overnight were administered with the interacting drugs in following order,

Group-II: Nifedipine (18 mg/kg B.W)

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Blood samples were collected at different time intervals and were estimated for blood glucose levels, serum Sitagliptin and serum insulin levels.

Stage-IV: After a washout period of six days, the same groups of rats which were fasted for overnight were administered with combination of interacting drugs and the TD of Sitagliptin as follows.

Group-II: Nifedipine followed by Sitagliptin after 30 minutes.

Blood samples were collected at different time intervals and were estimated for blood glucose levels serum Sitagliptin and serum insulin levels,

IV. RESULTS:

PHASE-III: PHARMACOKINETIC INTERACTIONS IN NORMAL RATS

STAGE-I: EFFECT OF VEHICLE ON BLOOD GLUCOSE LEVEL IN RATS (GROUP I, II):

The results of the blood glucose levels and the percent blood glucose reduction treated with vehicle were tabulated in the tables 3 and 3 (a) and were presented graphically in the fig.3 and 3(a). Reduction on blood glucose levels may be due to fasting of animals which deprived of both food and water.

STAGE-II: EFFECT OF SITAGLIPTIN (9 MG/KG B.W) ON BLOOD GLUCOSE LEVEL IN RATS (GROUP II):

Sitagliptin induced hypoglycaemia was studied by administering the TD of 9 mg/kg body weight to (Groups - II)in the actual laboratory conditions. TD of Sitagliptin produced -58.54±1.076%, -59.573±1.464%, -61.60±1.145% blood glucose change at 6 hr in (Groups- II) respectively. The results of effect of Sitagliptin on blood glucose level in rats Groups - II were given in the tables 3 and 3 (a) and were presented graphically in the fig.4 and 4(a).

STAGE-III, EFFECT OF NIFEDIPINE (18 MG/KG B.W) ON BLOOD GLUCOSE LEVEL IN RATS (GROUPS II):

The results of the blood glucose levels and the percent blood glucose reduction with Nifedipine alone, were tabulated in the tables 3 and 3(a) and were presented graphically in the fig 5 and 5(a)

The TD of Nifedipine shows percent blood glucose change of 118.76±2.240 % was observed at 2hr which was hyperglycaemic in group-II.

STAGE-IV, EFFECT OF NIFEDIPINE (18 MG/KG B.W FOLLOWED BY SITAGLIPTIN AFTER 30 MIN ON BLOOD GLUCOSE LEVEL IN RATS (GROUPS II):

The results of the blood glucose levels and the percent blood glucose reduction with Nifedipine followed by Sitagliptin after 30 min, were tabulated in the tables 3 and 3 (a) and were presented graphically in the fig.6 and 6(a).

It was observed that administration of Nifedipine followed by Sitagliptin after 30 min produced biphasic response with a peak response of percent blood glucose change of 77.53±3.076% and -37.01±2.807% at 2 hr and 6 hr intervals respectively. But compared to stage III hyperglycaemic effect produced by Nifedipine was reduced when administered in combination with Sitagliptin and also hypoglycaemic effects of Sitagliptin was reduced when compared to stage II when administered in combination with Nifedipine.

SERUM SITAGLIPTIN ESTIMATION RESULTS

EFFECT OF SITAGLIPTIN ON SERUM SITAGLIPTIN CONCENTRATIONS

The serum Sitagliptin levels and the pharmacokinetic parameters with TD of Sitagliptin are presented in the table-4 and table-7 the graphical presentation is given in fig 7

EFFECT OF NIFEDIPINE ON SERUM SITAGLIPTIN CONCENTRATIONS

The serum Sitagliptin levels and the pharmacokinetic parameters of Sitagliptin before and after treatment with Nifedipine are presented in the table-5 and table-8 the graphical presentation is given in fig- 7.

EFFECT OF NIFEDIPINE ON SERUM INSULIN LEVELS BY CHEMILUMINESCENCE ASSAY:

EFFECT OF SITAGLIPTIN ON SERUM INSULIN LEVEL:

The results are given in table-9. The average blood glucose levels were 67.30 ±0.536, 26.96 ±0.80, 45.07 ±0.69 at 0, 6, 10 hr respectively. The average insulin levels at the above corresponding intervals were 0.91±0.03, 2.60±0.10, 0.95 ±0.15 respectively.

EFFECT OF NIFEDIPINE ON SERUM INSULIN LEVEL:

The results are given in table-10 and table-11. From table-38 the average blood glucose levels were 77.43±0.94, 161.10±1.516, 77.14±0.967 at 0, 1, 10 hr respectively when administered alone. The average insulin levels at the above corresponding intervals were 0.76±0.12, 0.11±0.07, 0.70±0.22 respectively.

From table-11 the average blood glucose levels were 75.18±0.95, 156.21±1.514, 33.96±1.02 at 0,

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1, 10 hr respectively when administered along with Sitagliptin. The average insulin levels at the above corresponding intervals were 0.79±0.07, 0.10±0.08, 1.99±0.02 respectively.

V. DISCUSSION:

STUDIES ON THE INFLUENCE OF CALCIUM CHANNEL BLOCKERS-NIFEDIPINE ON THE PHARMACOKINETICS OF SITAGLIPTIN IN NORMAL RATS.

ESTIMATION OF SERUM SITAGLIPTIN BY UV-SPECTROSCOPY

This study was conducted in normal rats to validate the existence of the interaction between the selected drugs and Sitagliptin in rodent model and also to evaluate whether the interaction seen in rats was of pharmacodynamic or pharmacokinetic in nature. Sitagliptin produced hypoglycaemic effect with peak effect at 6 hr.

EFFECT OF NIFEDIPINE ON SERUM SITAGLIPTIN CONCENTRATIONS

The drug Nifedipine, a calcium channel blocker raised blood glucose level when administered alone and reduced the Sitagliptin response in normal & diabetic rats when administered in combination. It was observed that Nifedipine did not alter the serum Sitagliptin concentration. The study indicated that the interaction observed was pharmacodynamic interaction, which might be due to their opposing effects on insulin release as explained earlier.

ESTIMATION OF SERUM INSULIN BY CHEMILUMINESCENCE ASSAY

EFFECT OF NIFEDIPINE ON INSULIN RELEASE

The results with the TD of Nifedipine in normal and diabetic rats indicate that it raised the blood glucose levels when administered alone. It was also shown to reduce the hypoglycaemic effect produced by Sitagliptin. It did not alter the pharmacokinetic parameters of Sitagliptin in combination indicate that the interaction seen was pharmacodynamic interaction. The interaction occurred might be due to the effect of Nifedipine on the insulin release. Some authors also reported the antagonistic effect of Nifedipine on the insulin release which supports our results. The results of the present study indicates that the TD of Nifedipine decreased the insulin secretion at 2 hr compared to basal condition. Hence Nifedipine itself appears to produce hyperglycaemia.From the present studies, it was observed that insulin release after administration of TD of Nifedipine followed by Sitagliptin was less when compared to the results obtained from the TD by individual administration of Sitagliptin.

VI. CONCLUSION:

The aim of the present study was to find out the influence of Nifedipine / Amantadine on the pharmacokinetics and pharmacodynamics of Sitagliptin in rats. The rat model was used for both pharmacodynamic and pharmacokinetic interaction studies since it is most widely used species in drug metabolism and drug interaction studies. The influence of TD of Nifedipine / Amantadine on insulin release was studied in normal rats. TD of Sitagliptin produced hypoglycaemic effect in normal and diabetic rats. Nifedipine, a calcium channel blocker raised the blood glucose level in normal and diabetic rats when administered alone. It reduced the hypoglycaemic effect produced by Sitagliptin when administered in combination in normal and diabetic rats. Biphasic response was noticed when administered in combination with Sitagliptin. Initially hyperglycaemic effects later hypoglycaemic effects was observed. The pharmacokinetic parameters (serum Sitagliptin concentration) of Sitagliptin was not altered with treatment of Nifedipine. The TD of Nifedipine lowers the insulin levels in normal rats. Nifedipine appears to produce interaction with Sitagliptin by pharmacodynamic mechanism i.e, by the inhibition of intracellular influx of calcium ions inside the cell. Since the interaction was observed in rodent model. In future, studies can be extended to non-rodent models and also in clinical studies.

TABLE-3
PHASE-III: PHARMACOKINETIC INTERACTIONS IN NORMAL RATS.
STAGE-I: TABLE-3 BLOOD GLUCOSE LEVEL IN RATS TREATED WITH VEHICLE, SITAGLIPTIN, NIFEDIPINE AFTER WASHOUT PERIOD

Time(h)	Blood Glucose levels (mg / dl) in rats (Mean ± SEM)			
	Group-I(vehicle)	Group-II(Sitagliptin)	Group-II(Nifedipine)	Group-II(Nifedipine followed by

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				Sitagliptin)
0	67.68±0.472	64.94±1.201	70.91±0.852	70.52±0.626
1	66.80±0.328	57.83±1.264	124.48±1.672	118.25±1.748
2	65.54±0.711	49.66±0.729	155.14±2.721	125.2±2.169
3	64.75±0.343	40.07±0.403	142.21±2.846	93.18±4.861
4	63.21±0.323	37.60±0.914	113.3±1.506	88.81±1.805
6	62.46±0.283	26.92±0.306	99.87±2.935	44.41±1.979
8	61.92±0.198	33.03±1.035	94.26±2.243	50.65±2.167
10	61.74±0.804	46.25±1.247	88.84±2.136	64.35±1.895
12	60.52±0.700	59.96±1.383	80.15±3.896	66.96±1.204

TABLE-3(A) PERCENT BLOOD GLUCOSE CHANGE

Time(h)	Percent Blood Glucose change in rats (Mean ± SEM)			
	Group-I	Group-II	Group-III	Group-IV
0	-	-	-	-
1	-1.300±0.824	-10.948±1.636	75.53±2.357	69.23±2.479
2	-3.16±0.996	-23.528±0.841	118.76±3.837	77.53±3.076
3	-4.329±0.588	-38.29±0.533	100.53±4.013	32.13±6.893
4	-6.60±0.460	-42.100±1.224	59.77±2.124	25.93±2.56
6	-7.712±0.957	-58.54±1.076	40.83±4.138	-37.01±2.807
8	-8.510±0.638	-49.137±1.374	32.91±3.163	-28.17±3.072
10	-8.776±0.675	-28.786±1.664	25.27±3.013	-16.19±2.682
12	-10.57±0.1962	-7.660±1.846	13.02±5.494	-5.05±1.708

FIG-3
PHASE-I: PHARMACODYNAMIC INTERACTIONS IN NORMAL RATS
STAGE-I, BLOOD GLUCOSE LEVELS IN RATS TREATED WITH VEHICLE

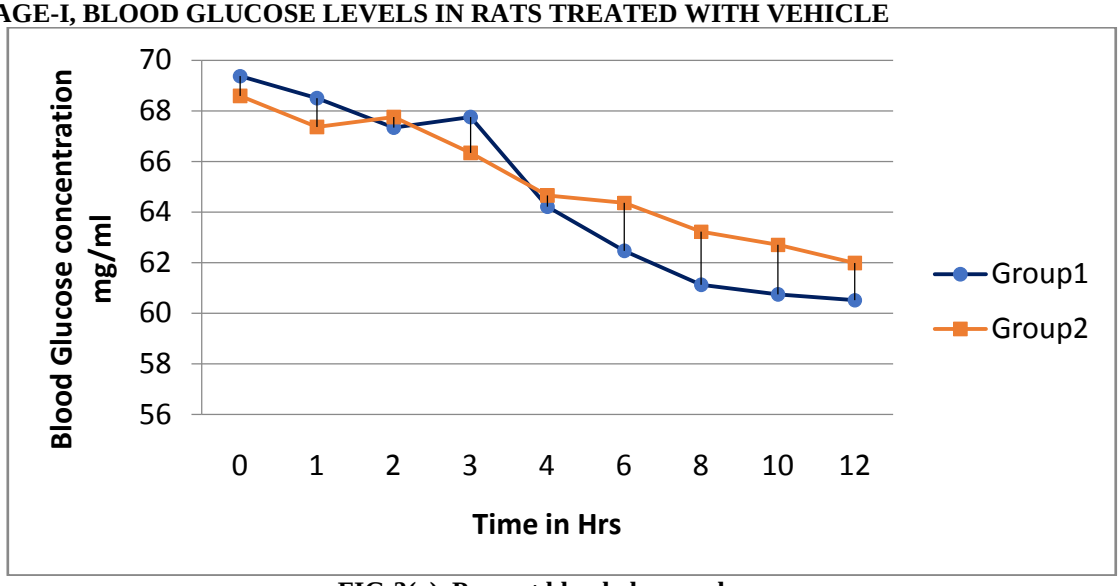

FIG-3(a). Percent blood glucose change

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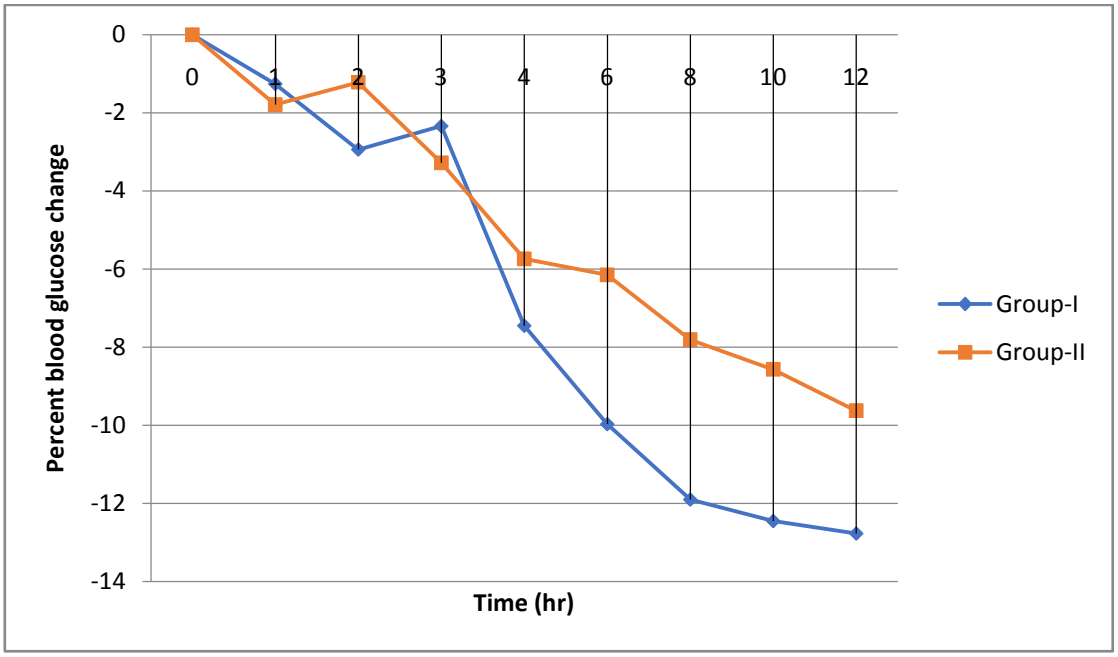

FIG-4. PHASE-III: PHARMACOKINETIC INTERACTIONS IN NORMAL RATS.

STAGE-II: EFFECT OF SITAGLIPTIN (9 MG/KG B.W) ON BLOOD GLUCOSE LEVEL IN RATS

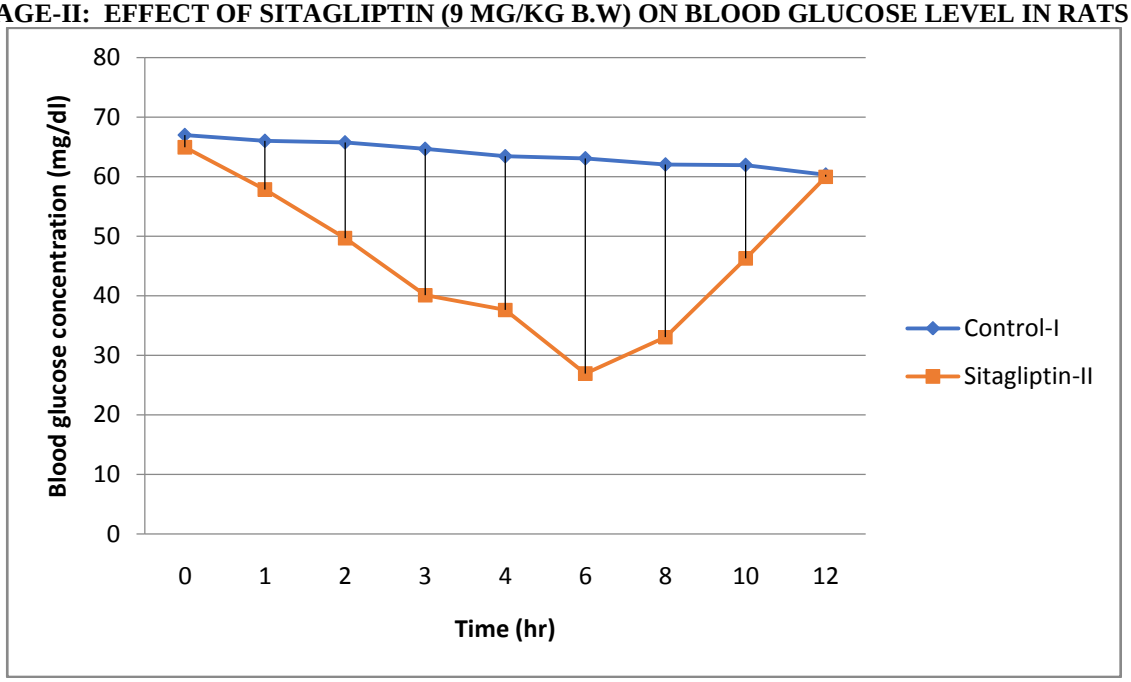

FIG. 4(A) - PERCENT BLOOD GLUCOSE CHANGE

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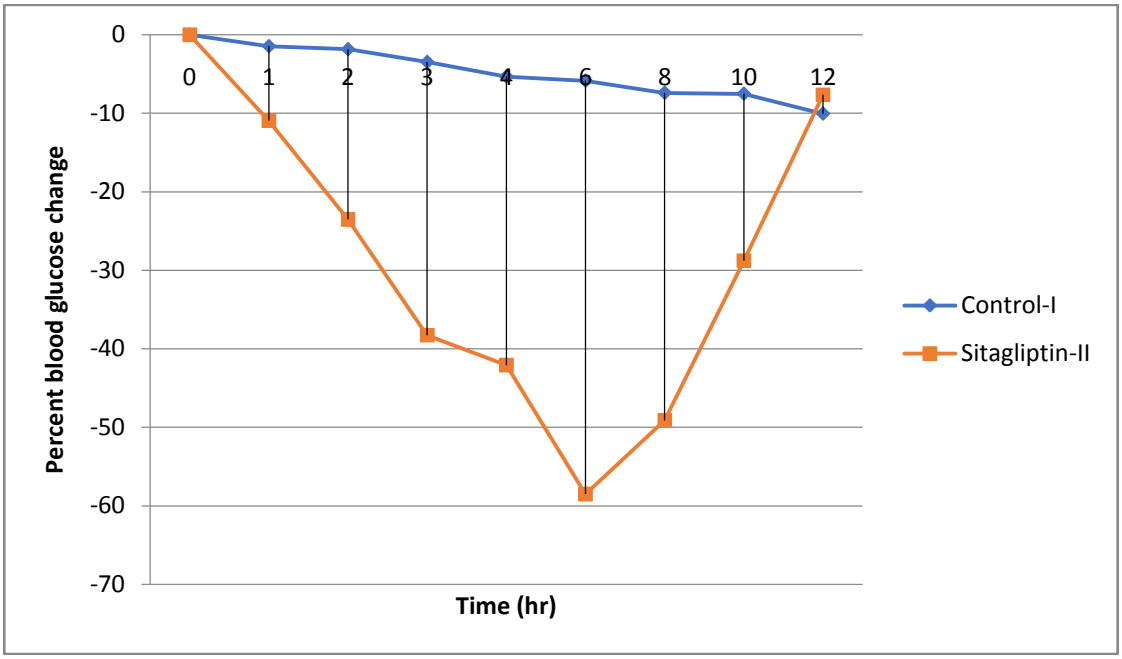

FIG-5. PHASE-III: PHARMACOKINETIC INTERACTIONS IN NORMAL RATS.

STAGE-III: EFFECT OF NIFEDIPINE (9 MG/KG B.W) / ON BLOOD GLUCOSE LEVEL IN RAT.

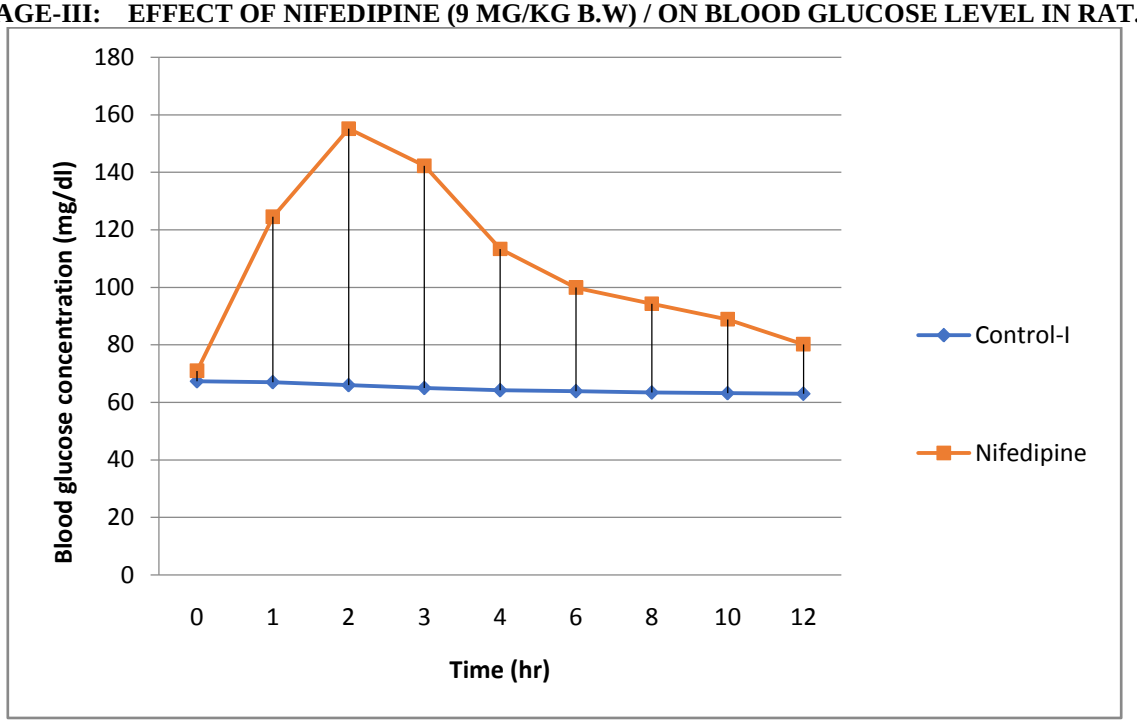

FIG. 5(A) - PERCENT BLOOD GLUCOSE CHANGE

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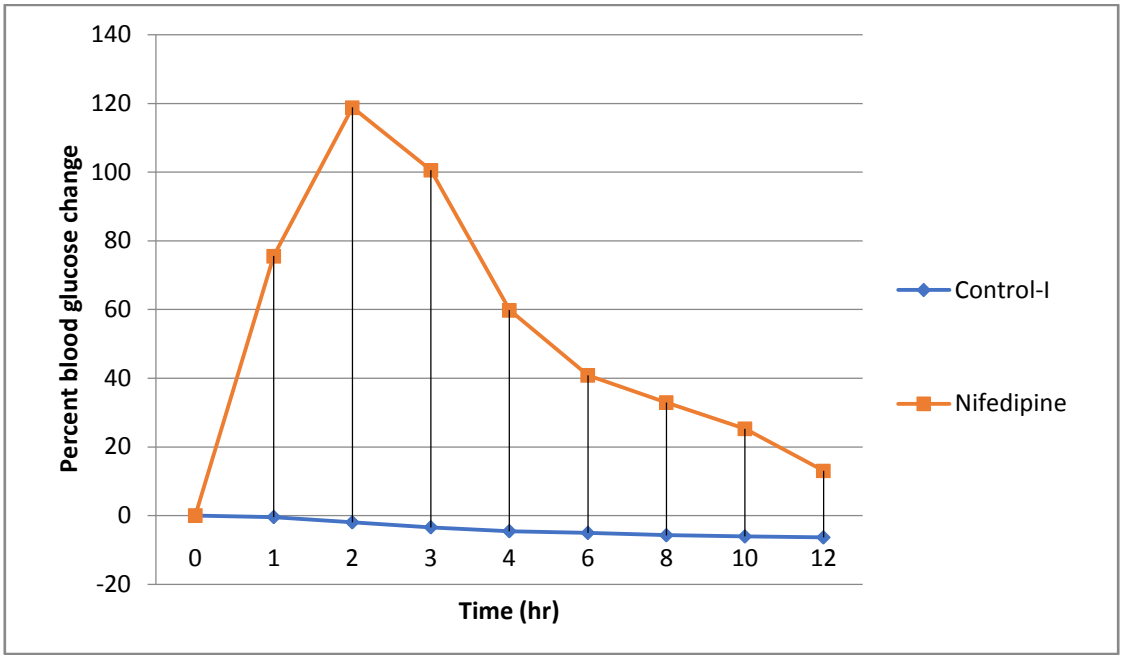

FIG-6. PHASE-III: PHARMACOKINETIC INTERACTIONS IN NORMAL RATS.

STAGE-IV: EFFECT OF NIFEDIPINE (9 MG/KG B.W) FOLLOWED BY SITAGLIPTIN AFTER 30 MIN ON BLOOD GLUCOSE LEVEL IN RATS.

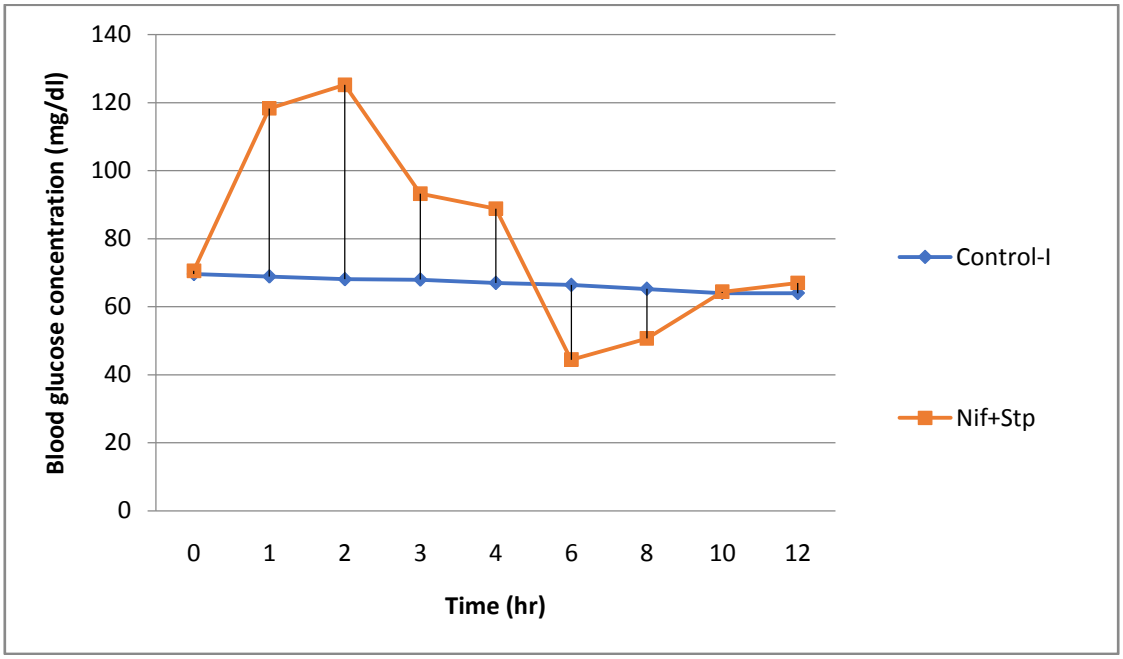

FIG. 6(A) - PERCENT BLOOD GLUCOSE CHANGE

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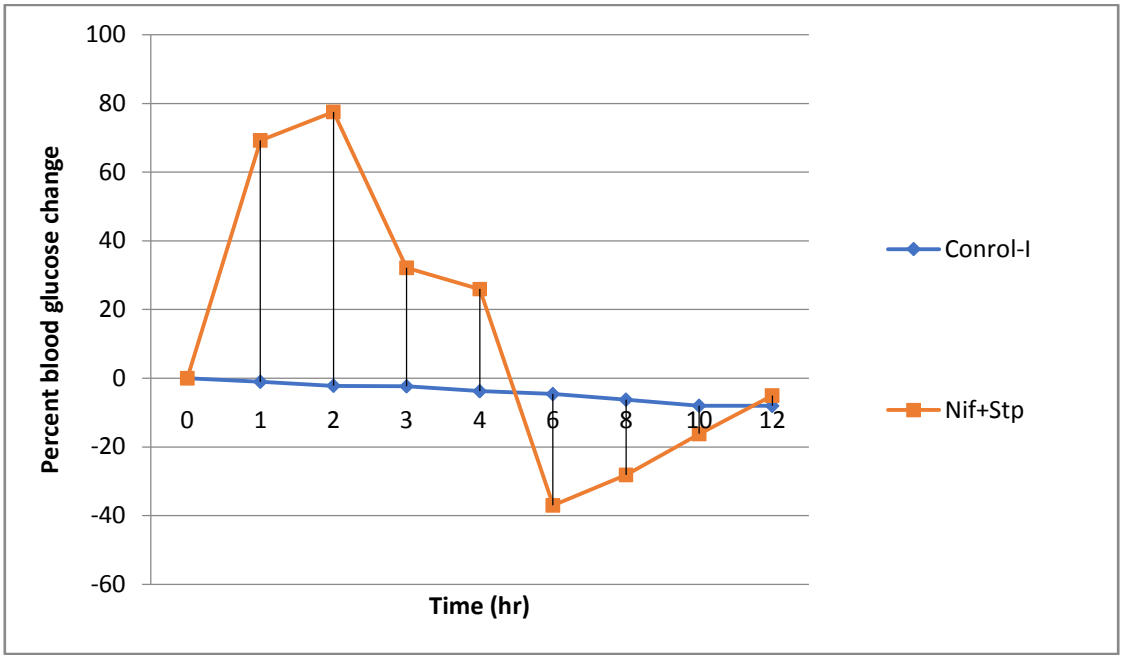

SERUM SITAGLIPTIN CONCENTRATION

TABLE-4. SERUM SITAGLIPTIN CONCENTRATIONS AFTER ORAL ADMINISTRATION OF SITAGLIPTIN TD IN NORMAL RATS (N=6)

Time(h)	Serum sitagliptin concentration (µg/ml)						Mean±SEM
	R_1	R_2	R_3	R_4	R_5	R_6	
0	0	0	0	0	0	0	0
1	0.096	0.093	0.094	0.097	0.094	0.095	0.094±0.006
2	0.132	0.181	0.164	0.173	0.157	0.148	0.159±0.072
3	0.586	0.59	0.598	0.583	0.587	0.591	0.589±0.021
4	0.991	0.983	1.024	1.076	1.018	1.050	1.023±0.014
6	1.795	1.824	1.896	1.763	1.732	1.745	1.792±0.024
8	1.420	1.469	1.456	1.483	1.412	1.435	1.445±0.011
10	1.101	1.093	1.084	1.075	1.037	1.068	1.076±0.092
12	0.891	0.844	0.812	0.835	0.873	0.828	0.847±0.012

TABLE-5. EFFECT OF NIFEDIPINE ON THE SERUM SITAGLIPTIN LEVELS IN NORMAL RATS (N=6)

Time(h)	Serum sitagliptin concentration (µg/ml)						Mean±SEM
	R_1	R_2	R_3	R_4	R_5	R_6	
0	0	0	0	0	0	0	0

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1	0.092	0.088	0.091	0.096	0.101	0.097	0.094±0.019
2	0.156	0.187	0.194	0.177	0.196	0.182	0.182±0.059
3	0.775	0.826	0.731	0.758	0.814	0.843	0.791±0.017
4	1.012	1.242	1.113	1.008	0.987	1.234	1.099±0.047
6	1.835	1.783	1.896	1.865	1.816	1.833	1.838±0.015
8	1.571	1.506	1.534	1.542	1.568	1.584	1.550±0.011
10	1.113	1.161	1.215	1.197	1.235	1.297	1.203±0.025
12	0.971	0.853	0.916	0.893	0.838	0.879	0.891±0.019

TABLE-6. EFFECT OF NIFEDIPINE FOLLOWED BY SITAGLIPTIN AFTER 30 MIN AND EFFECT OF SITAGLIPTIN ON THE SERUM SITAGLIPTIN LEVELS IN NORMAL RATS OF GROUPS (I, II) (MEAN ±SEM)

Time (h)	**Serum Sitagliptin levels (µg / ml) in rats (Mean±SEM)**	
	Group-I	**Group-II**
0	0	0
1	0.094±0.006	0.096±0.019
2	0.159±0.072	0.182±0.059
3	0.589±0.021	0.791±0.017
4	1.023±0.014	1.099±0.047
6	1.792±0.024	1.838±0.015
8	1.445±0.011	1.550±0.011
10	1.076±0.092	1.203±0.025
12	0.847±0.012	0.891±0.019

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FIG. 7 - EFFECT OF NIFEDIPINE FOLLOWED BY SITAGLIPTIN AFTER 30 MIN AND EFFECT OF SITAGLIPTIN ON THE SERUM SITAGLIPTIN LEVELS IN NORMAL RATS OF GROUPS (I, II) MEAN±SEM

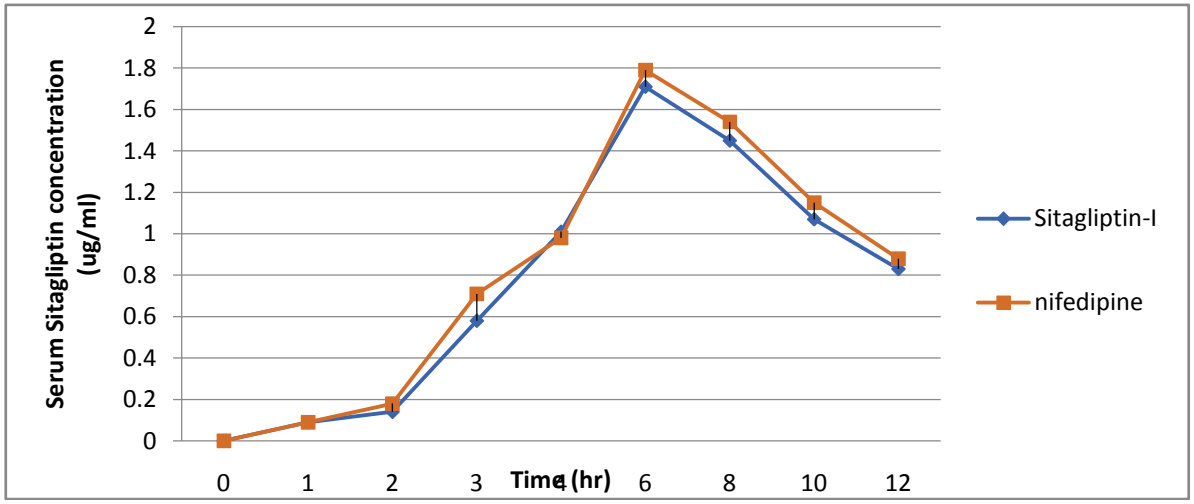

TABLE-7: PHARMACOKINETIC PARAMETERS OF SITAGLIPTIN TD IN NORMAL RATS (N=6)

Kinetic Parameter	R_1	R_2	R_3	R_4	R_5	R_6	Mean±SEM
AUC_{0-12} (µg /ml/h)	11.0435	11.9545	12.256	11.914	11.6	11.733	11.66±0.02
$AUMC_{0-12}$ (µg /ml/h*h)	141.0397	137.2318	134.0342	135.5953	136.5441	134.3596	135.47±0.33
K_e (h^{-1})	0.024369	0.029769	0.033268	0.290793	0.1982323	0.292963	0.00358±0.0004
$AUC_{0-\infty}$ (µg /ml/h)	19.46387	18.48375	17.87708	18.52409	21.74223	18.23912	17.75±0.125
$AUMC_{0-\infty}$ (µg /ml/h*h)	321.6607	266.0935	240.3991	267.2436	376.0801	263.5556	252.87±3.59
$T_{1/2}$ (h)	6.549178	5.361099	4.7973	5.485977	8.051051	5.445337	5.04±0.086
K_a (h^{-1})	0.768334	0.7683334	0.7683334	0.7683334	0.7683334	0.7683334	0.768±0
Clearance (ml/h)	69.35928	80.34086	80.55007	82.59517	72.43966	88.82009	136.08±5.85
Clearance (ml/h/kg)	462.3952	486.9143	503.438	485.854	413.9409	493.445	544.32±234.18
Vd_{ss}(ml)	1055.962	1052.028	978.3464	1084.086	1158.723	1167.851	1761.59±78.23
Vd_{ss}(ml/kg)	7039.743	6375.928	6114.666	6376.978	6621.273	6488.063	7046.38±312.95
Vd_{area}(ml)	655.4779	621.5228	557.6087	653.8458	841.5806	697.9153	989.87±46.53
Vd_{area}(ml/kg)	4369.853	3766.805	3485.055	3846.152	4809.032	3877.307	3959.48±186.14
MRT (h)	16.5260	14.39608	13.44734	14.42682	17.29722	14.45002	14.24±0.10

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	4						
C_{max} (µg/ml)	1.795	1.824	1.896	1.763	1.732	1.745	1.71±0.009
T_{max} (h)	6	6	6	6	6	6	6.00±0.00

TABLE-8 PHARMACOKINETIC PARAMETERS OF SITAGLIPTIN TD AFTER TREATMENT WITH TD OF NIFEDIPINE IN NORMAL RATS (N=6)

***** Significant at P< 0.001; ** Significant at P< 0.01; * Significant at P< 0.05, compared to sitagliptin control (Table- 33)**

Kinetic Parameter	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**Mean±SEM**
AUC_{0-12} (µg /ml/h)	9.933001	9.723009	9.891511	9.644002	9.111667	9.211248	9..24±0.057
$AUMC_{0-12}$(µg /ml/h*h)	20.71303	21.635208	21.011789	21.138924	20.424311	21.201789	21.70±3.046
K_e (h^{-1})	0.660635	0.667144	0.696866	0.614366	0.660572	0.638925	0.660032±0.0001
$AUC_{0-\infty}$ (µg /ml/h)	10.25358	10.72226	10.99754	10.18314	11.01753	10.25021	10.78±0.442
$AUMC_{0-\infty}$ (µg /ml/h*h)	25.677	25.8981	26.4109	27.4926	24.7809	26.3942	26.78±11.77
$T_{1/2}$ (h)	2.4147754	2.876397	2.376078	2.789652	2.424312	2.4706892	2.48011±0.1996
K_a (h^{-1})	2.305	2.768	2.768	2.676	2.768	2.768	2.768±0.00
Clearance (ml/h)	131.6614	145.9138	157.5083	164.44915	174.92702	162.46673	165.12±2.762
Clearance (ml/h/kg)	877.7425	880.7113	865.0553	869.162	899.5135	867.5273	856.49±11.048
Vd_{ss}(ml)	272.5864	251.1623	262.5079	274.073	268.1002	277.2211	273.93±6.37
Vd_{ss}(ml/kg)	1817.242	1894.765	1950.052	1878.183	1880.668	1788.524	1836.40±16.67
Vd_{area}(ml)	458.7772	541.2153	523.7083	518.393	578.3557	592.1978	564.68±14.93
Vd_{area}(ml/kg)	3058.515	3382.596	3491.388	3435.516	3189.038	3175.47	3458.75±59.72
MRT (h)	2.504199	2.166812	2.52223	2.25692	2.4747	2.68267	2.26±0.100
C_{max} (µg/ml)	1.835	1.783	1.896	1.865	1.816	1.833	1.879±0.008
T_{max} (h)	6	6	6	6	6	6	6.00±0.00

GLUCOSE AND INSULIN PARAMETERS

TABLE-9. SERUM INSULIN (µIU/ML) AND BLOOD GLUCOSE LEVELS (MG/DL) BEFORE AND AFTER TREATMENT WITH SITAGLIPTIN IN NORMAL RATS (N=4)

Rat	**Body weight (gm)**	**Time (hr)**					
		0hr		**6hr**		**10 hr**	
		Blood Glucose (mg/dl)	**Serum Insulin (µI**	**Blood Glucose (mg/dl)**	**Serum Insulin (µIU / ml)**	**Blood Glucose (mg/dl)**	**Serum Insulin (µIU / ml)**

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			U / ml)				
R1	200	67.45	0.98	27.32	2.32	45.15	0.99
R2	200	67.72	0.83	26.44	2.59	44.89	0.97
R3	225	65.47	0.96	25.14	2.66	43.45	1.00
R4	150	68.58	0.89	28.97	2.84	46.82	0.93
Mean ± SEM		67.30±0.536	0.91 ± 0.03	26.96± 0.80	2.60± 0.10	45.07± 0.69	0.97± 0.15

TABLE-10 SERUM INSULIN (µIU/ML) AND BLOOD GLUCOSE LEVELS (MG/DL) BEFORE AND AFTER TREATMENT WITH NIFEDIPINE IN NORMAL RATS (N=4)

Rat	**Body weight (gm)**	**Time (hr)**					
		0hr		**2 hr**		**8hr**	
		Blood Glucose (mg/dl	**Serum Insulin (µIU / ml)**	**Blood Glucose (mg/dl)**	**Serum Insulin (µIU / ml)**	**Blood Glucose (mg/dl)**	**Serum Insulin (µIU / ml)**
R1	180	79.60	0.78	165.58	0.12	79.89	0.65
R2	200	78.42	0.73	162.72	0.10	77.65	0.68
R3	225	75.63	0.65	158.39	0.13	74.54	0.62
R4	175	76.09	0.90	157.73	0.10	76.50	0.87
Mean ±SEM		77.43± 0.94	0.76± 0.12	161.10± 1.516	0.11± 0.07	77.14± 1.11	0.70± 0.22

TABLE-11. SERUM INSULIN (µIU/ML) AND BLOOD GLUCOSE LEVELS (MG/DL) BEFORE AND AFTER TREATMENT IN COMBINATION WITH NIFEDIPINE AND SITAGLIPTIN IN NORMAL RATS (N=4)

Rat	**Body weight (gm)**	**Time (hr)**					
		0hr		**2 hr**		**6 hr**	
		Blood Glucose (mg/dl)	**Serum Insulin (µIU / ml)**	**Blood Glucose (mg/dl)**	**Serum Insulin (µIU / ml)**	**Blood Glucose (mg/dl)**	**Serum Insulin (µIU / ml)**
R1	175	77.60	0.72	151.06	0.10	31.32	1.99
R2	175	74.42	0.63	159.75	0.10	33.44	1.97
R3	200	75.63	0.85	156.36	0.11	35.14	2.00
R4	190	73.09	0.98	157.70	0.10	35.97	2.03

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Mean ± SEM	75.18± 0.95	0.79± 0.07	156.21± 1.514	0.10± 0.08	33.96± 1.02	1.99± 0.02

ABBREVATIONS

DM Diabetes mellitus
DPP4Dipeptidyl peptidase-4
GLP-1glucagon –like peptide-1
GIP glucose- dependent insulinotropic polypeptide
GLUT2glucose transporter 2
VDCC voltage-dependent (Calcium) Ca++ channel
KV voltage-dependent K+ channel
cAMPcyclic adenosine mono phosphate
ATP/ADPAdenosine-triphosphate/ Adenosine-diphosphate
PKA protein kinase A
B.W body weight
GOD/POD Glucose oxidase–peroxidase
CNS central nervous system
CPCSEA **Committee for the Purpose of Control And Supervision of Experiments on Animals**
TD therapeutic dose

REFERNCES

[1]. Cerveny JD, Leder RD &Weart CW. Issues surrounding tight glycaemic control in people with type 2 diabetes mellitus. Annpharmacother 1998;32(9):869-905.DOI: 10.1345/aph.17375

[2]. Aaron Vinik& Mark Flemmern. Diabetes and macrovascular disease. Journal of Diabetes and Its Complications. 2002; 16: 235–45.DOI: 10.1016/s1056-8727(01)00212-4

[3]. Gallwitz B. Sitagliptin: Profile of a novel DPP-4 inhibitors for the treatment of type 2 diabetes.Drugs today 2007;43: 13-25.DOI: 10.1358/dot.2007.43.1.1043909

[4]. Drug Information Centre (DIC), Bangalore, Karnataka.

[5]. J.Girard. The Incretins: From the concept to their use in treatment of type 2 diabetes. Part A: Incretins: Concept & Physiological functions. Diabetes & Metabolism 2008; 34: 550-59.DOI: 10.1016/j.diabet.2008.09.001

[6]. Beate Ritz, Shannon L. Rodes. L-type calcium channel blockers and parkinson's disease in Denmark. Ann Neurol. 2010 May 67(5): 600-606.

[7]. Ramesh. S, Lokesh .B, Rupali .J, and Prashanth . L. Validated spectrophotometric methods for simultaneous estimation of paracetamol, domperidone and tramadol HCL in pure and tablet dosage form. J. Clin. Metrol. 2010;4(1):21-27.

[8]. Laurence DR, Bacharach AL. Evaluation of drug activities and pharmacometrics. London and New York: Academic press ;1964.

[9]. Viana G.S, Medeiros A.C, Lacerda A.M, Leal L.K, Vale T.G, Matos F.J. Hypoglycaemic and anti-lipemic effects of aqueous extract from Cissus sicyoides. BMC Pharmacol, 2004;8: 4-9.DOI: 10.1186/1471-2210-4-9

[10]. Kim D.Wang L, Beconi M, Eiermann GJ, Fisher MH, He H ,and Lyson SK(2005). (2R)-4-oxo-4-[3-(tri fluoromethy)-5,6-dihydrol 1,2,4]triazolo[4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluoro phenyl) butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of Type 2 Diabetes.J MedChem 48;141-51. DOI: 10.1021/jm0493156

[11]. Dinesh K Badyal, Jasleen kaur. Sitagliptin: A new class of oral drug for type 2 diabetes. 2008;10(2).

[12]. A Ramachandran, AK Das, SR Joshi, CS Yajnik, S Shah, KM Prasanna kumar. Current status of diabetes in India and need for novel therapeutic agents. Supplement to JAPI, vol 58, 2010.

[13]. Laurence DR, Bacharach AL. Evaluation of drug activities and pharmacometrics. London and New York: Academic press ;1964.

[14]. Viana G.S, Medeiros A.C, Lacerda A.M, Leal L.K, Vale T.G, Matos F.J. Hypoglycaemic and anti-lipemic effects of aqueous extract from Cissus sicyoides. BMC Pharmacol, 2004;8: 4-9.DOI: 10.1186/1471-2210-4-9