



## A PKPD INTERACTION BETWEEN *MOMORDICA CHARANTIA* AND ORAL HYPOGLYCEMIC DRUG –SITAGLIPTIN IN STZ INDUCED HYPERGLYCEMIC RATS

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### ABSTRACT

Rural people are still dependent on indigenous knowledge for health care that are being influenced by culture and socioeconomic aspects, providing a cheaper and accessible alternative to the high cost pharmaceutical remedies. In spite of the overwhelming influence and our dependence on modern medicine and tremendous advances in synthetic drugs, many people still rely on herbs the reason is that, if the herbs are used properly they don't have any side effects. Hence, the research studies required to be subjected to pharmacodynamic and pharmacokinetic studies in order to determine effect of *Momordica charantia* herb on the hyperglycaemic patients who are taking the therapy with synthetic drugs. This study was to discover the influence of *Momordica charantia* on the pharmacokinetics and pharmacodynamics of sitagliptin and in rats. Results have proved the negative (decrease) effect of *Momordica charantia* on pharmacokinetics but positive (increase) effect on pharmacodynamics of Sitagliptin.

### INTRODUCTION

Diabetic Mellitus (Hyperglycemia) is an endocrine disease and not a single disease which is a group of chronic metabolic or heterogeneous affliction due to the irregular secretions of insulin and action of insulin or both. Absence or reduced insulin in turn leads to abnormal high blood sugar level and glucose intolerance [1-5]. Sitagliptin prolongs the activity of proteins that increase the release of insulin after blood sugar rises, such as after a meal. Sitagliptin is a selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4), which metabolizes the naturally occurring

incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) resulting in enhanced glucose-dependent insulin secretion from the pancreas and decreased hepatic glucose production. Since GLP-1 enhances insulin secretion in the presence of raised blood glucose levels, inhibiting DPP-IV activity will increase and prolong the action of GLP-1 by reducing its rate of inactivation in plasma. Sitagliptin reduces hemoglobin A1c (HbA1c), fasting and postprandial glucose by glucose-dependent stimulation of insulin secretion and

inhibition of glucagon secretion . GLP-1 has other widespread effects including delaying gastric emptying, significantly reducing glucagons levels and possible central effects on the appetite. In clinical trials, Sitagliptin demonstrated an overall incidence of side effects comparable to placebo. The most common side effects in studies were upper respiratory tract infection, stuffy or running nose, sore throat, headache and diarrhea. The incidence of hypoglycemia with Sitagliptin monotherapy was not significantly different than placebo. Pooled data from 2 monotherapy and 2 combination trials show that the incidence of hypoglycemia was 1.2% and 0.9% for Sitagliptin 100mg and placebo respectively [6-9].

*Momordica charantia*(*M. charantia*), commonly referred to as bitter gourd, karela and balsam pear. Its fruit is also used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa. Abundant pre-clinical studies have documented in the anti-diabetic and hypoglycaemic effects of *Momordica charantia* through various postulated mechanisms. However, clinical trial data with human subjects are limited and flawed by poor study design and low statistical power. The present review is an attempt to highlight the antidiabetic activity as well as phytochemical and pharmacological reports on *M. charantia* and calls for better-designed clinical trials to further elucidate its possible therapeutic effects on diabetes<sup>[10]</sup>

There is scope for the potential herb-interactions between *Momordica charantia* and Sitagliptin. This can cause few adverse reactions as a result, it precipitates potentially life-threatening effects. Hence, the study need to be subjected to pharmacological studies in order to discover their effect on the patients who are taking the treatment with synthetic drugs.

## **MATERIALS AND METHODS**

### **DRUGS AND CHEMICALS**

Adult Albino rats weight between 150±20grams (Mahavear enterprises Hyderabad, Telangana.) were used in this

experimental study. These animals were acclimatized to standard laboratory's conditions of suitable temperature (27°C ± 1°C) and maintained on 12:12 hours light: dark cycle in animal's house. They were maintained in elevated rat's wire cages and provided with regular rat's chow (Standard pellets contains diet – Balaji life sciences, Hyderabad, Telangana.), distilled water *ad-libitum* for 14 days. These experimental protocols were in conducted according with IAEC/ CPCSEA.

### **Preparation of crude extract:**

The fresh fruits of *M charantia* (Bitter melon) were purchased from the local market. The fruits were then washed thoroughly with tap water and cut into thin slices. The sliced pieces were dried completely under the mild sun and grinded with an electric grinder into coarse powder and used for cold extraction. After extraction the yield was found to be about 35g/1kg bitter melon powder. The authenticity of *M. charantia* was identified by a plant taxonomist from the Department of Botany, University of Sathavahana University<sup>[11]</sup>

### **Pre treatment**

Albino rats were selected for this study (150±20grams), animals were maintained under the suitable conditions in animal house. [IAEC-1322/po/s/10/CPCSEA].The rats were kept in the animal cages and high fatty food and water are supplied in the form of carbohydrates: proteins: fat in 42:18:40.for 14days.

### **Induction of Hyperglycemia in Rats by streptozotocin {60mg/kg}**

After 15 days of feeding with highly fatty food the rats were fasted for a period of 18hrs before the induction of hyperglycemia & singledose administration of the 60 mg/kg of Streptozocin (Sigma Aldrich; St. Louis; MO; USA) were injected intra-peritonally (freshly dissolve in the normal saline solution). After STZ administration, the animals were free accessed with food (pellet diet) & water. moderate polydipsia and marked polyuria were observed in diabetic hyperglycemic rats. After three days i.e. after 72hrs of injection, fasting blood glucose concentration was determined by

following glucose levels by using commercial glucose estimation kits with UV-Visible Spectrophotometer at 505nm based on the oxidase/peroxidase GOD/POD method. The Rats showing the fasting blood glucose level more than 150 mg/dL were considered the hyperglycaemic-rats and selected for the different grouping in the experimental design.

**Experimental Study Design** - The hyperglycemic rats were divided into 6 groups 6 animals in each.

I Group: Diabetic control

II Group: Low dose of *Momordica charantia* (100mg/Kg)

III Group: High dose of *Momordica charantia* (500mg/Kg)

IV Group: Oral hypoglycemic drug- 100mg/Kg of Sitagliptin.

V Group: Combination of 50mg/Kg of Sitagliptin + 500mg/Kg of *Momordica charantia*

VI Group: Combination of 100mg/Kg of Sitagliptin + 500mg/Kg of *Momordica charantia* <sup>[12]</sup>.

### **Pharmacokinetics study in hyperglycemic rat model:**

#### **Single dose Study**

These pharmacokinetic studies were carried out in hyperglycaemic rats (weight b/n 180grams and 250grams). These animals were housed in animal's wire cages with free access to diet and water *ad-libitum*. The overnight fasting rats were divided into 6 different groups (n=6) and followed the treatment mentioned in the study design. Blood samples were collected at predetermined intervals of 0hr, 1hr, 2hr, 4hr, 8hr, 12hr and 24hr in the form of microcentrifugal tubes containing Na<sup>+</sup> citrate from retro-orbital puncture under diethyl ether anaesthesia. The blood samples were subjected to centrifugation at 3000rpm per 10minutes and plasma was stored at -20°C for analysis and estimation of kinetic parameters as AUC 0 - ∞, C<sub>max</sub>, k<sub>a</sub>, k<sub>e</sub>, CL/F, T<sub>max</sub>, V/F, AUC 0-t & t<sub>1/2</sub>.

#### **Multiple dose study**

The hyperglycemic rats were divided into 6 different treatment groups same as mentioned in study design and daily treatment is carried for 21 days.

Samples of blood were collected from different rat's groups on 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> day immediately after drug treatment. Samples of blood are collected into microcentrifugal tubes containing Na<sup>+</sup> citrate from retro-orbital puncture under anaesthesia. These blood samples were subjected to centrifugation at 3000rpm per 10 minutes and plasma was stored at -20°C for analysis and estimation of kinetic parameters as AUC 0 - ∞, V/F, k<sub>a</sub>, C<sub>max</sub>, CL/F, T<sub>max</sub>, k<sub>e</sub>, AUC 0-t & t<sub>1/2</sub>.

### **Pharmacodynamics study in the hyperglycaemic rats**

#### **Single dose study**

In this study, treatment was given to all groups of animals as per experimental design. Pharmacodynamic parameters like urea, glucose and cholesterol levels were estimated at the interval of 0, 1, 2, 4, 8, 12 and 24 hours by UV spectrophotometer.

#### **Multiple dose study**

In this study, daily treatment was given to all groups of animals for 3 weeks as per experimental design. Pharmacodynamic parameter like urea, cholesterol and glucose levels are estimated at the time interval of 0, 7, 14 and 21 days by UV spectrophotometer.

#### **Statistical Application**

ANOVA followed by Dunnett test was performed for comparison between different groups of animals. *P* value fewer than 5% (*P* < 0.05) was considered statistically significant. All clinical data were expressed in the form of Mean ± Sd. Pharmacokinetics data was calculated by using *pk solver* software and statistical analysis and graphical representations were done by *Graph pad prism-9.0*.

#### **Histopathological Study**

After estimation of last blood glucose level, the animals were sacrificed and histopathological studies to estimate the inflammation and necrosis related changes in pancreas. The pancreatic tissues were stained using H&E stains and observed under resolution 100x.

**RESULTS:**

**Table No.1 Blood glucose levels mg/dL (0<sup>th</sup>,1<sup>st</sup>,2<sup>nd</sup>,4<sup>th</sup>,8<sup>th</sup>, 12<sup>th</sup> and 24<sup>th</sup> Hour) after oral administration of *Momordica charantia*, Sitagliptin and combination of Sitagliptin and *Momordica charantia* in diabetic rats (n=6).**

TREATMENT/ HOURS	BLOOD GLUCOSE LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	500mg/kg	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
0 <sup>th</sup> Hour	401 ± 0.89	412.66 ± 0.81*	393.16 ± 0.75**	385.16 ± 0.75**	383.16 ± 0.75**	373.83 ± 0.75**
1 <sup>st</sup> Hour	415.17 ± 0.75	417 ± 0.63*	366.83 ± 0.75**	364.67 ± 0.82**	349.5 ± 0.55**	346.17 ± 0.75**
2 <sup>nd</sup> Hour	412.5 ± 0.55	348 ± 0.63*	328 ± 0.63**	328.67 ± 0.52**	314.33 ± 0.82**	301 ± 0.63**
4 <sup>th</sup> Hour	413.33 ± 1.03	337 ± 0.63*	318.33 ± 0.52**	315.17 ± 0.75**	306.33 ± 0.52**	288.17 ± 0.41**
8 <sup>th</sup> Hour	412 ± 0.63	299.33 ± 1.03*	277.17 ± 0.41**	274.5 ± 0.55**	275.83 ± 0.75**	270.67 ± 0.82**
12 <sup>th</sup> Hour	411 ± 0.63	318.67 ± 0.52*	285.5 ± 0.55**	267.5 ± 0.55**	244.83 ± 0.75**	242.17 ± 0.75**
24 <sup>th</sup> Hour	411.17 ± 0.75	324.17 ± 0.41*	315.83 ± 0.75**	275 ± 0.63**	250.5 ± 1.05**	244.17 ± 0.41**

**Table No.2 Blood cholesterol levels mg/dL (0<sup>th</sup>,1<sup>st</sup>,2<sup>nd</sup>,4<sup>th</sup>,8<sup>th</sup>, 12<sup>th</sup> and 24<sup>th</sup> Hour) after oral administration of *Momordica charantia*, Sitagliptin and combination of Sitagliptin and *Momordica charantia* in diabetic rats (n=6).**

TREATMENT/ HOURS	BLOOD CHOLESTEROL LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	500mg/kg	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
0 <sup>th</sup> Hour	201.37 ± 0.86	207.34 ± 0.9	204.9 ± 0.84	207.18 ± 0.76**	198.62 ± 0.53**	193.05 ± 0.73**
1 <sup>st</sup> Hour	203.6 ± 0.8	201.4 ± 0.51	197.35 ± 0.83	192.18 ± 0.81**	180.17 ± 0.75**	183.62 ± 0.54**
2 <sup>nd</sup> Hour	204.35 ± 0.51	188.19 ± 0.76	184.36 ± 0.54	170.14 ± 0.92**	168.17 ± 0.9**	163.18 ± 0.75**
4 <sup>th</sup> Hour	207.15 ± 0.73	178.8 ± 0.85	172.46 ± 0.93	158.92 ± 0.77**	152.29 ± 0.68**	145.19 ± 0.76**
8 <sup>th</sup> Hour	208.33 ± 0.35	148.02 ± 0.89	145.2 ± 0.73	147.52 ± 1.13**	135.19 ± 1.01**	138.06 ± 0.91**
12 <sup>th</sup> Hour	212.18 ± 0.72	158.02 ± 0.61	159.11 ± 0.85	145.57 ± 0.9**	133.19 ± 0.75**	128.14 ± 0.63**
24 <sup>th</sup> Hour	216.41 ± 0.48	178.02 ± 0.89	170.33 ± 1.57	134.65 ± 0.82**	130.15 ± 0.94**	125.19 ± 0.76**

*Values are given as mean ± Standard deviation.*

*\*\*Statistical significance p < 0.001 (compared with the control group)*

*\*Statistical significance p < 0.05 (compared with the control group)*

*MM- Momordica charantia*

*n - number of animals used*

**Table No.3 Blood urea levels mg/dL (0<sup>th</sup>,1<sup>st</sup>,2<sup>nd</sup>,4<sup>th</sup>,8<sup>th</sup>, 12<sup>th</sup> and 24<sup>th</sup> Hour) after oral administration of *Momordica charantia*, Sitagliptin and combination of Sitagliptin and *Momordica charantia* in diabetic rats (n=6).**

TREATMENT/ HOURS	BLOOD UREA LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	500mg/kg	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
0 <sup>th</sup> Hour	63.8 ± 0.82	63.9 ± 0.73	73.19 ± 0.75	78.23 ± 0.75	67.82 ± 0.74	65.84 ± 0.85
1 <sup>st</sup> Hour	65.42 ± 0.63	63.19 ± 0.77	68.84 ± 0.75	65.17 ± 1.09	63.2 ± 0.55	56.2 ± 0.62
2 <sup>nd</sup> Hour	67.15 ± 0.67	62.23 ± 0.71	65.19 ± 0.75	64.19 ± 0.74	53.19 ± 0.56	51.16 ± 0.88
4 <sup>th</sup> Hour	70.85 ± 0.98	61.17 ± 0.75	64.19 ± 0.76	61.2 ± 0.76	48.16 ± 0.52	46.18 ± 0.8
8 <sup>th</sup> Hour	72.28 ± 0.95	58.18 ± 0.64	45.8 ± 0.97	48.15 ± 0.89	44.3 ± 0.79	40.83 ± 0.74
12 <sup>th</sup> Hour	71.92 ± 0.98	61.19 ± 0.75	57.89 ± 0.65	48.28 ± 0.79	41.16 ± 0.96	38.56 ± 0.56
24 <sup>th</sup> Hour	72.83 ± 0.75	62.84 ± 0.8	58.18 ± 0.61	38.2 ± 0.73	39.16 ± 0.87	34.64 ± 0.71

**Table No.4 Blood glucose levels mg/dL (0<sup>th</sup>,7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day) after oral administration of *Momordica charantia*, Sitagliptin and combination of Sitagliptin and *Momordica charantia* in diabetic rats (n=6).**

TREATMENT/ DAYS	BLOOD GLUCOSE LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	500mg/kg	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
0 <sup>th</sup> Day	413.39 ± 1.02	415.91 ± 0.68	391.19 ± 0.75	402.19 ± 0.85	382.19 ± 0.74	371.18 ± 0.58
7 <sup>th</sup> Day	391.91 ± 0.67	234.82 ± 0.74	234.9 ± 0.82	218.03 ± 0.61	215.18 ± 0.93	196.52 ± 1.12
14 <sup>th</sup> Day	386.42 ± 0.81	182.11 ± 0.66	152.82 ± 1.03	152.94 ± 1.02	128.02 ± 0.64	129.11 ± 0.69
21 <sup>st</sup> Day	391.14 ± 1.06	135.91 ± 0.7	124.61 ± 0.49	114.03 ± 0.91	115.04 ± 0.86	112.68 ± 0.82

**Table No.5 Blood cholesterol levels mg/dL (0<sup>th</sup>,7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day) after oral administration of *Momordica charantia*, Sitagliptin and combination of Sitagliptin and *Momordica charantia* in diabetic rats (n=6).**

TREATMENT/ DAYS	BLOOD CHOLESTEROL LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	500mg/kg	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
0 <sup>th</sup> Day	192.72 ± 0.97	188.6 ± 0.73	185.88 ± 0.97	184.96 ± 0.84	180.66 ± 1.03	176.02 ± 1.04
7 <sup>th</sup> Day	193.43 ± 0.52	103.77 ± 0.85	104.15 ± 0.82	116.95 ± 0.84	98.13 ± 0.9	92.17 ± 0.9
14 <sup>th</sup> Day	191.7 ± 0.6	87.15 ± 0.76	86.3 ± 0.74	85.93 ± 0.53	76.3 ± 0.96	78.31 ± 0.75
21 <sup>st</sup> Day	187.32 ± 0.66	76.43 ± 0.84	67.08 ± 0.91	68.07 ± 0.96	61.89 ± 0.74	56.64 ± 0.76

**Table No.6 Blood urea levels mg/dL (0<sup>th</sup>,7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day) after oral administration of *Momordica charantia*, Sitagliptin and combination of Sitagliptin and *Momordica charantia* in diabetic rats (n=6).**

TREATMENT/ DAYS	BLOOD UREA LEVELS (mg/dL)					
	DIABETIC CONTROL	MM (DOSE)		SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	500mg/kg	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
0 <sup>th</sup> Day	68.12 ± 0.34	65.27 ± 0.68	67.12 ± 0.67	64.3 ± 0.72	68.89 ± 0.38	57.17 ± 0.75
7 <sup>th</sup> Day	75.1 ± 0.66	45.1 ± 0.54	40.14 ± 0.52	34.88 ± 0.73	32.62 ± 0.83	26.89 ± 0.78
14 <sup>th</sup> Day	78.63 ± 0.36	35.18 ± 0.63	34.86 ± 0.3	29.04 ± 0.95	23.98 ± 0.87	22.64 ± 0.84
21 <sup>st</sup> Day	81.33 ± 0.71	31.25 ± 0.56	29.46 ± 1.11	23.2 ± 0.84	18.89 ± 0.78	17.19 ± 0.75

*Values are given as mean ± Standard deviation.*

*\* \*Statistical significance p < 0.01 (compared with the control group)*

*\*Statistical significance p < 0.05 (compared with the control group)*

*MM- Momordica charantia n - number of animals used.*

**Table No.7 Mean plasma Sitagliptin concentrations (µg/ml) (Single dose study).**

TREATMENT/ DAYS	PLASMA CONCENTRATIONS (µg/ml)			
	DIABETIC CONTROL	SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
1 <sup>st</sup> Hour	0	39.46±0.044	19.13±0.031	36.19±0.052
2 <sup>nd</sup> Hour	0	61.35±0.012	31.24±0.028	59.03±0.039
4 <sup>th</sup> Hour	0	53.68±0.052	16.11±0.026	46.15±0.031
8 <sup>th</sup> Hour	0	24.71±0.031	10.61±0.029	19.68±0.081
12 <sup>th</sup> Hour	0	9.11±0.082	8.73±0.029	8.14±0.052
24 <sup>th</sup> Hour	0	0	0	0

**Table No.8 Mean plasma Sitagliptin concentrations (µg/ml) (Multiple dose study).**

TREATMENT/ DAYS	PLASMA CONCENTRATIONS (µg/ml)			
	DIABETIC CONTROL	SITAGLIPTIN (DOSE)	SITAGLIPTIN + M M (DOSE)	
	Vehicle	100mg/kg	50mg/kg +500mg/kg	100mg/kg +500mg/kg
0 <sup>th</sup> Day	0	39.45±0.022	16.22±0.019	35.18±0.043
7 <sup>th</sup> Day	0	62.15±0.031	34.63±0.029	58.04±0.018
14 <sup>th</sup> Day	0	31.16±0.054	19.22±0.018	42.23±0.026
21 <sup>st</sup> Day	0	14.94±0.021	11.82±0.031	14.52±0.063

**Table No.9 Effect of *Momordica charantia* on Pharmacokinetic parameters of Single dose administration of Sitagliptin in diabetic rats (n=6).**

Pharmacokinetic parameter	Units for Pharmacokinetic parameters	100mg/kg of SITAGLIPTIN	SITAGLIPTIN + <i>Momordica charantia</i> (DOSE)	
			50mg/kg+500mg/kg	100mg/kg+500mg/kg
ka	h <sup>-1</sup>	1.642±0.19	1.013±0.33	1.319±0.44
ke	h <sup>-1</sup>	0.135±0.08	0.114±0.02	0.129±0.02
t <sub>1/2</sub>	h	12.41±0.63	12.16±0.55	12.38±0.46
V/F	(mg/kg)/(µg/ml)	62.19±0.022	51.38±0.43	59.03±0.033**
CL/F	(mg/kg)/(µg/ml)/h	0.89±0.003	0.91±0.11	0.94±0.003
Tmax	h	2.01 ±0.16	1.68±0.83	1.86±0.28*
Cmax	µg/ml	816.19±0.039	735.12±0.052**	809.16±0.042**
AUC 0-t	µg/ml*h	7134.08±0.26	6813.54±0.84**	7098.32±0.14*
AUC 0 - ∞	µg/ml*h	7217.05±0.044	7019.15±0.18**	7106.52±0.23

**Table No.10 Effect of *Momordica charantia* on Pharmacokinetic parameters of Multiple dose administration of Sitagliptin in diabetic rats (n=6)**

Pharmacokinetic parameter	Units for Pharmacokinetic parameters	100mg/kg of SITAGLIPTIN	SITAGLIPTIN + <i>Momordica charantia</i> (DOSE)	
			50mg/kg+500mg/kg	50mg/kg+500mg/kg
ka	h <sup>-1</sup>	1.714±0.22	1.018±0.46	1.328±0.51
ke	h <sup>-1</sup>	0.134±0.06	0.113±0.03	0.125±0.03
t <sub>1/2</sub>	h	12.19±0.82	12.11±0.42	12.16±0.35
V/F	(mg/kg)/(µg/ml)	61.27±0.019	52.91±0.38	58.01±0.046**
CL/F	(mg/kg)/(µg/ml)/h	0.88±0.004	0.92±0.15	0.97±0.004
Tmax	h	2.02 ±0.19	1.64±0.86	1.71±0.26*
Cmax	µg/ml	825.18±0.037	729.18±0.049**	806.19±0.028**
AUC 0-t	µg/ml*h	7165.05±0.32	6863.28±0.76**	7085.25±0.18*
AUC 0 - ∞	µg/ml*h	7183.05±0.039	7023.28±0.16**	7128.56±0.44

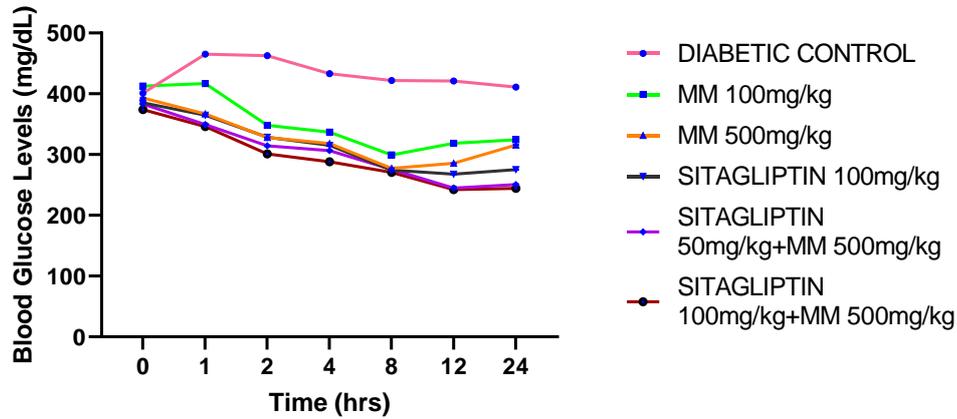
Values are given as mean ± Standard deviation.

\* \*Statistical significance  $p < 0.01$  (compared with the control group)

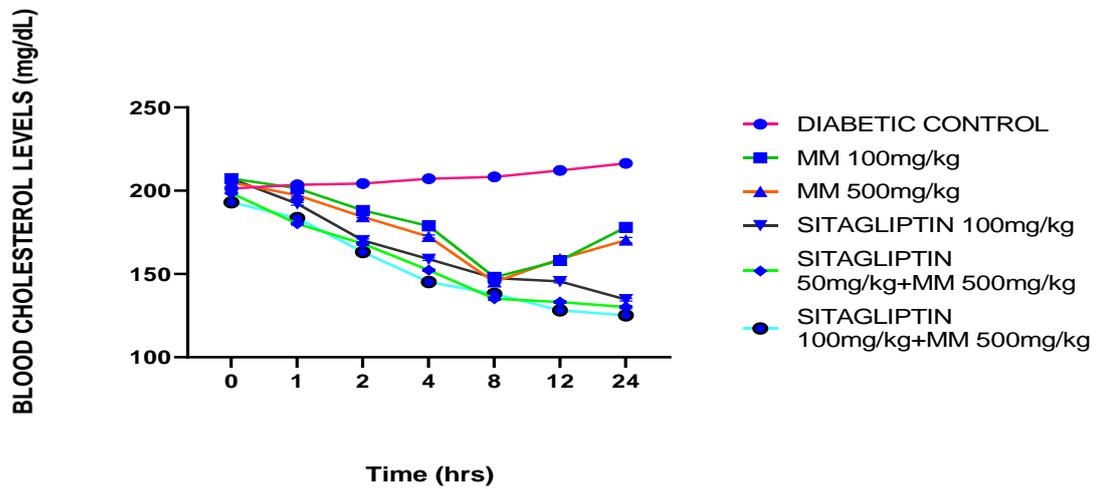
\*Statistical significance  $p < 0.05$  (compared with the control group)

MM- *Momordica charantia* n - number of animals used.

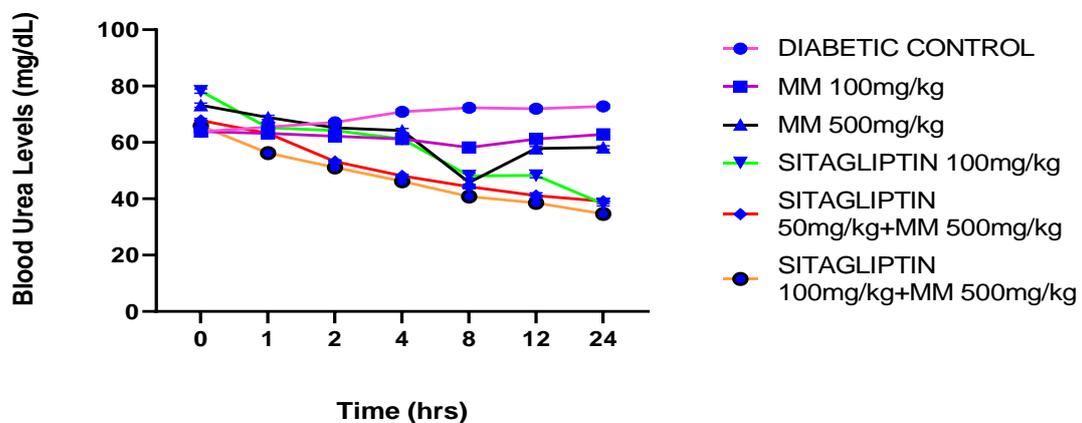
Graph.1: Blood glucose levels mg/dL (0th,1st ,2nd,4th,8th, 12th and 24th Hour) after oral administration of Momordica charantia, Sitagliptin and combination of Sitagliptin and Momordica charantia in diabetic rats (n=6).



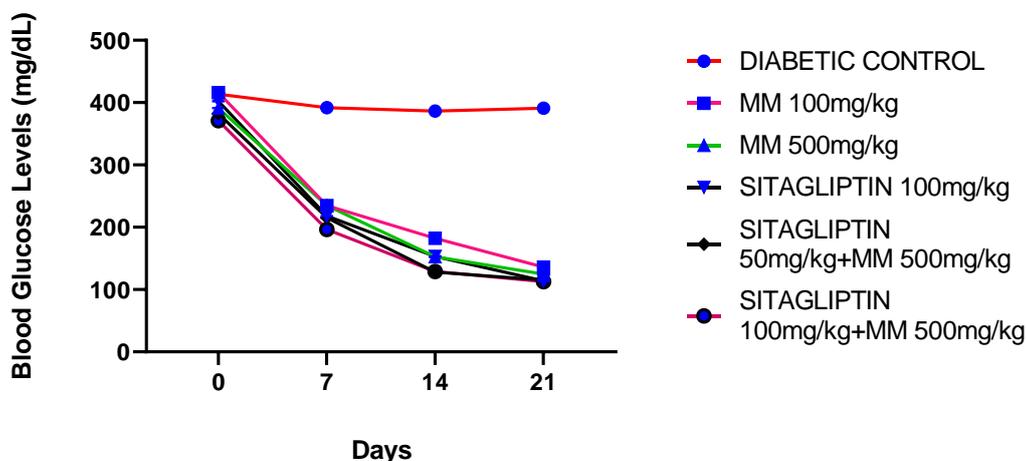
Graph.2 Blood cholesterol levels mg/dL (0th,1st ,2nd,4th,8th, 12th and 24th Hour) after oral administration of Momordica charantia, Sitagliptin and combination of Sitagliptin and Momordica charantia in diabetic rats (n=6)



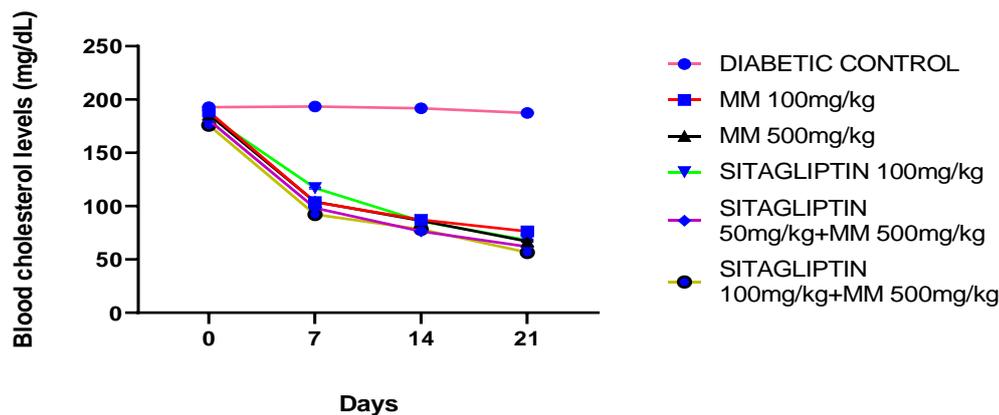
Graph.3 Blood urea levels mg/dL (0th,1st ,2nd,4th,8th, 12th and 24th Hour) after oral administration of Momordica charantia, Sitagliptin and combination of Sitagliptin and Momordica charantia in diabetic rats (n=6).



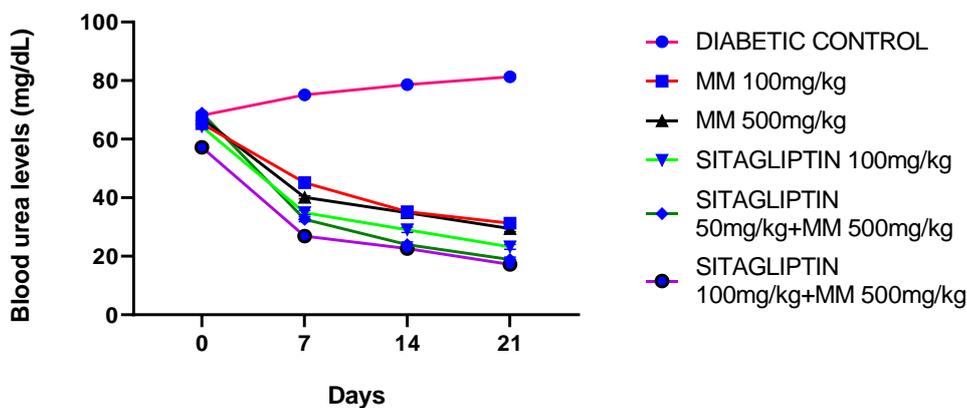
**Graph.4 Blood glucose levels mg/dL (0th,7th, 14th and 21st day) after oral administration of Momordica charantia, Sitagliptin and combination of Sitagliptin and Momordica charantia in diabetic rats (n=6).**



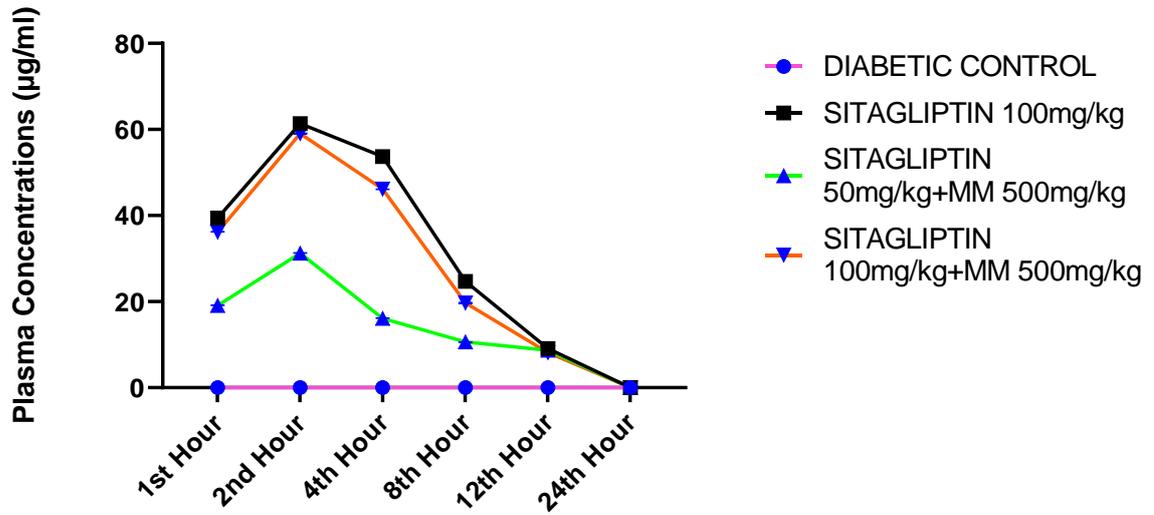
**Graph.5 Blood cholesterol levels mg/dL (0th,7th, 14th and 21st day) after oral administration of Momordica charantia, Sitagliptin and combination of Sitagliptin and Momordica charantia in diabetic rats (n=6).**



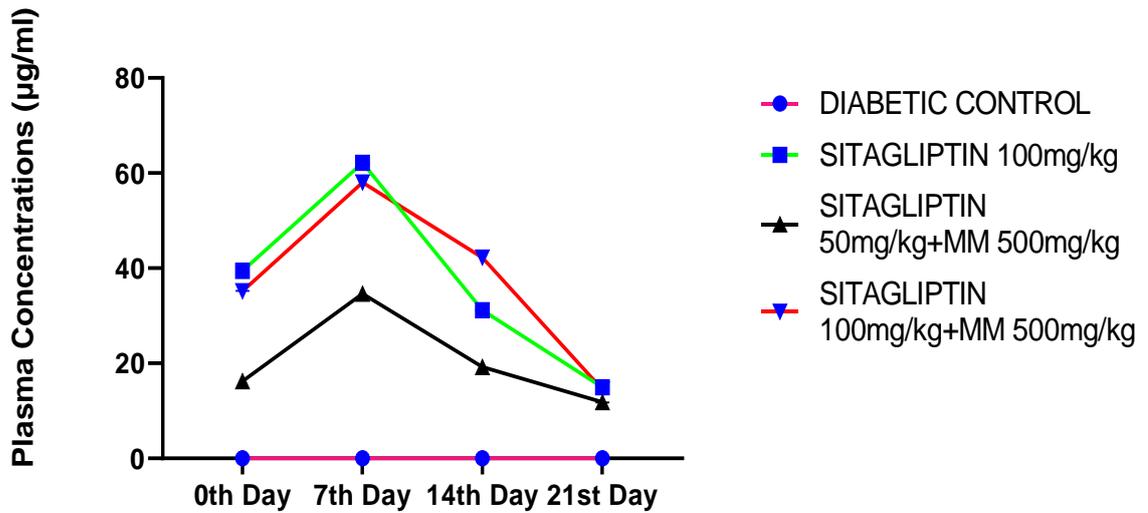
**Graph.6 Blood urea levels mg/dL (0th,7th, 14th and 21st day) after oral administration of Momordica charantia, Sitagliptin and combination of Sitagliptin and Momordica charantia in diabetic rats (n=6).**



Graph.7 Mean plasma Sitagliptin concentrations ( $\mu\text{g/ml}$ ) (Single dose study)



Graph.8 Mean plasma Sitagliptin concentrations ( $\mu\text{g/ml}$ ) (Multiple dose study)



## DISCUSSION:

### Pharmacodynamic study:

The combination of high dose of Sitagliptin (100 mg/kg) with 500mg/kg *Momordica charantia* showed maximum hypoglycemic action, decrease in serum cholesterol and urea levels. The effect produced by combination of Sitagliptin (50 mg/kg) with *Momordica charantia* was greater than the hypoglycaemic action produced by *Momordica charantia* (500 mg/kg) alone and Sitagliptin (10 mg/kg).

### Pharmacokinetic study:

The Single dose study showed that, 97.25% decrease in AUC( 0 -  $\infty$ ) in 500mg/kg of *Momordica charantia* and 50mg/kg of Sitagliptin. 98.46% decrease AUC ( 0 -  $\infty$ ) in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin. C max was decreased by 90.07% in 500mg/kg of *Momordica charantia* and 50mg/kg of Sitagliptin, 99.14% in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin in single dose study.

Significant decrease in absorption rate constant Ka by about 61.69% in Lower dose of 500mg/kg of *Momordica charantia* and 50mg/kg of Sitagliptin, 80.32% in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin. Significantly increase in clearance 2.24% in 500mg/kg of *Momordica charantia* and 50mg/kg of Sitagliptin. 5.61% in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin compared to 100mg/kg Sitagliptin.

The multiple dose study shows that, 97.72% decrease in AUC( 0 -  $\infty$ ) in 500mg/kg of *Momordica charantia* and 50mg/kg of Sitagliptin. 99.23% decrease AUC( 0 -  $\infty$ ) in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin. C max was decreased by 88.36% in 500mg/kg of *Momordica charantia* and 50mg/kg of

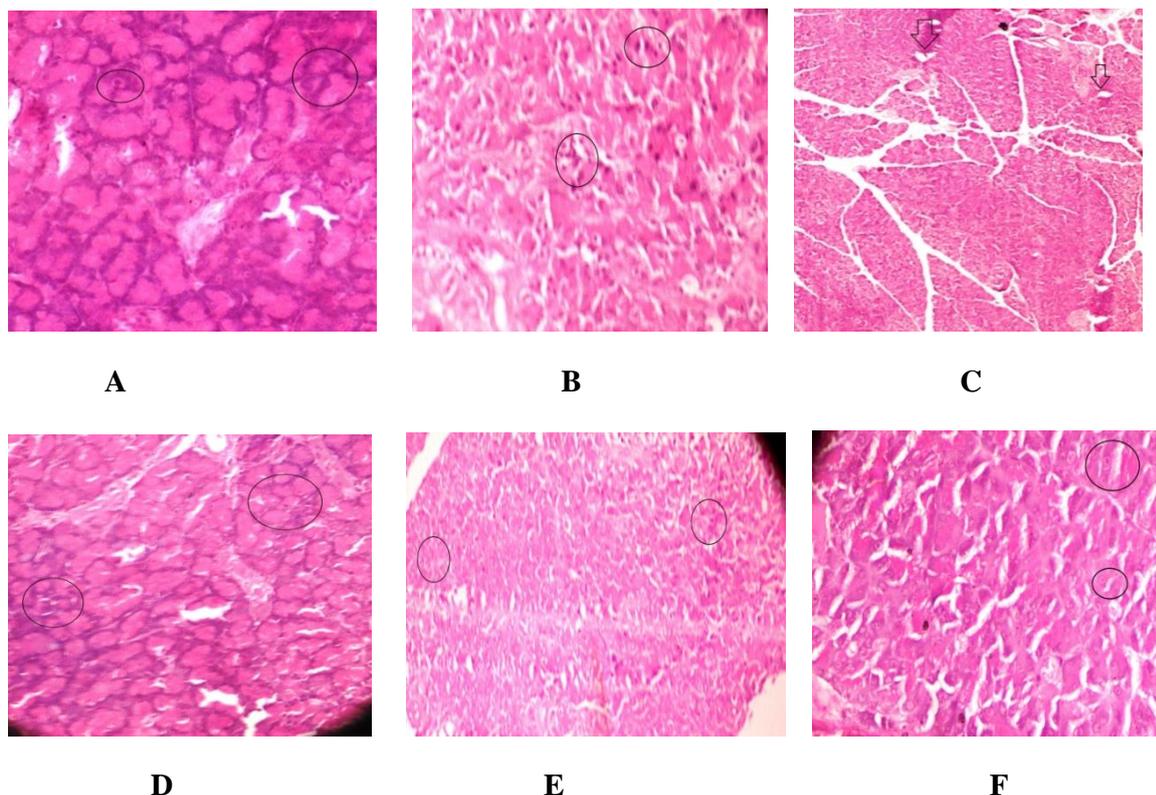
Sitagliptin, 97.69% in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin in multiple dose study. Significant decrease in absorption rate constant Ka by about 59.39% in Lower dose of 500mg/kg of *Momordica charantia* and 50mg/kg of Sitagliptin, 77.47% in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin. Significantly increase in clearance 4.54% in 500mg/kg of *Momordica charantia* and 50mg/kg of Sitagliptin. 10.22% in 500mg/kg of *Momordica charantia* and 100mg/kg of Sitagliptin compared to 100mg/kg Sitagliptin.

The exact reason behind the reduction in pharmacokinetic parameters was unknown but, it was understood that the combination of *Momordica charantia* extract with Sitagliptin in fact reduces exposure of the synergic drugs without reducing the pharmacodynamic activity. The proposed combination allows a safe therapy with less adverse effects.

### Histological study:

The histological study shows that the combination therapy (Sitagliptin + *Momordica charantia*) involved in the increase the number of islets and recovered the partially damaged  $\beta$  cells in pancreas when compare to the Individual treatment.

Slide A shows that pancreatic cells were damaged due to development of diabetes from STZ. Figure B shows that few pancreatic cells were damaged due to Sitagliptin. Figures C,D,E,F shows that  $\beta$  cells are regenerated in pancreatic tissue. Normal  $\beta$ -cells were observed in low and high doses of Sitagliptin and *Momordica charantia*. (Slides: D&F). In the Sitagliptin group more damaged  $\beta$ -cells as compared with the 500mg of *Momordica charantia* +100 mg of Sitagliptin and 500mg of *Momordica charantia* +50mg of Sitagliptin (Figures: B,C&E).



**H&S Staining of Pancreatic islets of Diabetic Control, *Momordica charantia* alone, Sitagliptin alone and combination of *Momordica charantia* & Sitagliptin treated Diabetic Rats. A. diabetic control , B. 100mg of *Momordica charantia* C. 500mg of *Momordica charantia*, D.100mg of Sitagliptin.E.500mg of *Momordica charantia*. +50mg of Sitagliptin, F. 500mg of *Momordica charantia* +100mg of Sitagliptin**

Histopathological studies revealed that the volume of islet cells in pancreas was significantly more in drug treated animals compared to the Diabetic control. The islet cells were shrunken and lytic cellular changes were observed in Diabetic control, Individual treatment had improved it but combination groups with a higher dose of Sitagliptin showed the return of islets close to original cytoarchitecture. In combination group, islets were big and cells were clear with good vascular pattern. The results of combination group with a high dose of Sitagliptin produced increment to the volume of islets in pancreas compared to individual treatment. In this study, *Momordica charantia* was decrease the absorption and increase the clearance of Sitagliptin. Hence care must be taken when the combination is taken by diabetic patients.

**CONCLUSION:** The above herb-drug interaction reveals that combination of Sitagliptin (100 mg/kg) with 500mg/kg of *Momordica charantia* have hypoglycemic action significantly as compared to the Sitagliptin (50 mg/kg) with *Momordica charantia* (500 mg/kg) and Sitagliptin (100 mg/kg) alone in pharmacodynamic studies. Combination of Sitagliptin (100 mg/kg) with 500mg/kg of *Momordica charantia* have increased AUC, C max , absorption rate constant  $K_a$  and decreased clearance as compared to the Sitagliptin (50 mg/kg) with *Momordica charantia* (500 mg/kg) in pharmacokinetic studies.

**Ethical issue:**

All experimental process of animals was approved by the Institutional Animal Ethical Committee of SRR college, of pharmacy.

### **Conflict of interest**

The authors declare that there are no conflicts of interest.

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