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Research paper

Influence of temsirolimus on antidiabetic effect of sitagliptin in experimental animal

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Abstract

The present study was carried out to evaluate the drug interaction between an antihyperglycemic agent and anticancerous drug,In healthy and Dexamethasone induced diabetic rats. The blood samples were collected up to 24 h and blood glucose level was estimated. In healthy albino rats Pretreatment with Temsirolimus (6.75 mg/kg for seven days) has significantly altered the onset of hypoglycemia (15.50+1.47 to21.40±.54), p< 0.001 at the 2nd hour significantly enhanced the peak hypoglycemia (29.70+.60 %) before treatment to (44.47+1.21 %) after treatment(p<0.001) at 4th hour (i.e. 18.71±.38 to 20.43±.89, p<0.001 induced by Sitagliptin. Whereas in Dexamethasone treated rats, pretreatment with Temsirolimus (6.75 mg/kg) for 7 days has decreased the onset of hypoglycemia, significantly enhanced the peak hypoglycemia (24.13+.6417%) before treatment to (45.62+2.845 %) after treatment(P<.001) at the 8th hour. This study indicates that therapeutic drug monitoring has been required for therapeutic doses of temsirolimus and antidiabetic drugs when used concomitantly.

Highlights

1) Influence of Temsirolimus on hypoglycemic activity of Sitagliptin in healthy rats.
2) Influence of Temsirolimus on hypoglycemic activity of Sitagliptin in Dexamethasone induced hyperglycemic rats.
3) To suggest the alteration in the dose and frequency of administration of Oral antidiabetic agents when they are used along with Temsirolimus.

Abbreviations

P.o.- per os ‘by mouth’, DM-Diabetes mellitus,GOD-Glucose oxidase (GOD), POD- Peroxidase (POD).

Keywords: *Influence of Temsirolimus; Antidiabetic Effect of Sitagliptin; Dexamethasone and Drug Interaction.*

1. Introduction

The incidence of type 2 diabetes mellitus and cancer is increasing globally. The complexity of these diseases is difficult to elucidate. The hypothesis that increases the risk of diabetes and certain cancer is difficult to correlate. Further complexities arise from evidences that particular drugs used in the treatment of diabetes influence cancer projection. Herein, we review the evidences from studies that have addressed these relationships and summarize the effect of temsirolimus on sitagliptin and vice versa frequently encountered in this paper.A drug interaction is a state in which usually another drug affects the activity of a "drug when both are administered simultaneously. Cancer is the most commonly diagnosed incidence in mortality all over the world. In recent years drug interactions, diagnosis and treatment had made a certain degree of progress, but Cancer is still a very important public health problem in the world. Thus, early diagnosis, effective treatment and analysis prognosis were of great significance to reducing the Cancer mortality. To guide decision-making for therapeutic strategies for Cancer patients and improve their prognosis, a better understanding of the relevant factors affecting Cancer prognosis is urgently needed.

Diabetes mellitus (DM) is one of the commonest chronic and metabolism diseases. An estimated 290 million people worldwide had diabetes mellitus, and the number of Diabetes Mellitus suffers will rise to 450 million by 2032, approx 7.5% of the total adult population.

The concurrence of Diabetes Mellitus with the increasing encumbrance of cancer worldwide has created interest in significant epidemiological and biological associations between these medical conditions. DM can seriously affect quality of life. DM can not only cause neurological and vascular complications, but is also closely related to the occurrence, development and prognosis of cancer. At present, surplus clinicians are seeing whether patients have writhed from diabetes during the treatment of cancer, and diabetologists often have to cope up diabetes in patients who are being treated for cancer. Insulin confrontation or compensatory hyperinsulinemia leads to metabolic alterations, and is convoluted in the development of the microenvironment for tumor progression.

However, it is unclear whether cancer patients with DM are more likely to receive a worse cancer prognosis compared to patients without DM. A meta-analysis has reported the effect of DM on cancer prognosis (Walker JJ et al. 2013, Tong L et al. 2014, Cos M A & Flórez J et. Al 1997 and Bapat S P 2015). Diabetes mellitus is a polygenic disease with abnormally high glucose intensities in the blood; numerous metabolic disorders apparent by excessive urination and tenacious thirst. Diabetic patients may also be affected with many other diseases like hypertension, cardiovascular diseases and peptic ulcer, fungal infections, etc. which require prolong treatment. It is reported that hypertension is prone to develop with patients having diabetes. In such cases multiple drug, therapy is needed to prescribe. So, agents like Sitagliptin and antidiabetic agent like temsirolimus are administered concomitantly (Klil-Drori A J et al. 2017, Madkhali A A et al. 2015, Tabernero J et al. 2011, Balkan B et al. 1999, Holst J J & Deacon CF 2004, Matsuo T & Odaka H 1992).

There are reports that sitagliptin is known to get metabolized through Cytochrome P-450 enzyme system and it is an inducer of this system, hence there is a possibility of occurrence of pharmacokinetic type of drug interactions with concomitantly used drugs (Torre L A et al. 2012, Kasichayanula S et al. 2011, Bavec A 2014). Sitagliptin metabolized by Cytochrome P-40 enzyme system. Therefore, the present study was carried out on healthy and diabetic rats to assess the influence of sitagliptin on the antidiabetic effects of temrolimus (Tornio A et al 2012, Patel CG et al. 2011 and Levetan C 2007).

2. Materials and methods

The studies were carried out in the Department of Pharmacology in our institution which is duly licensed by the CPCSEA (committee for the Purpose of Control and Supervision of Experiments in Animals). The study protocols were approved according to current regulations of CPCSEA by the Institution Animal Ethics Committee for studies in experimental animals.

All the animals (rats) used in the study were procured from Mahavir Enterprises, Hyderabad. Registration number 346/CPCSEA and were housed under standard husbandry conditions in the institutional animal house. Hence, the same may be considered as source of animal procurement in the subsequent sections. A total of 50 rats (either sex) were selected for the current study.

1) Sitagliptin: A solution was prepared by using 2% w/v gum acacia as a suspending agent to represent 6 mg/ml.
2) Temsirolimus: The suspensions of Temsirolimus were prepared in 2% gum acacia to represent 1mg/ml.
3) Albino Rats: Obtained from Mahavir Enterprises, Hyderabad
4) Glucose estimation Kit (Pathozyme diagnostic kit).
5) Motor and pestle, alcohol, low voltage electric lamp, micropipette (5-50µl), 1 ml graduated pipettes, epindroff tubes, thin Aluminium foil, incubator and double distilled water, etc.
6) Semi autoanalyzer (RMSBCA-201).
7) Weighing balance (5mg-350gm) by shimatzu

2.1. Drugs

Sitagliptin was obtained from Abhishek Chemicals LTD Gujarat. Temsirolimus was obtained from Sigma Aldrich. Temsirolimus (10 mgkg^{-1}, P.O.) suspensions were prepared using 2% w/v gum acacia as suspending agent.

2.2. Experimental procedure

In healthy Rats Six albino rats of either sex weighing between 1.5-1.8 kg were randomly selected for the study. They were marked suitably for ready identification. The animals were housed in colony cages under standard husbandry conditions. On the previous day of experimentation, the food was withdrawn 18-hrs advance. However, water was allowed ad libitum. The fasting was continued till the completion of the experiment. Next day, the blood samples were withdrawn from the marginal ear vein (0.5 ml, each) for determination of basal glucose concentration. Then the animals were administered with plain 2% w/v acacia suspension of Temsirolimus (volume matched with the average volume of drugs administrated in the subsequent studies). Thereafter the blood samples Next day, the blood samples were withdrawn from the marginal ear vein (0.5 ml, each) for determination of basal glucose concentration. were collected at 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hours and analyzed for the determining the glucose concentration using GOD/POD method and expressed as mg dL^{-1} of blood.

In the first part of this study, the hypoglycemic effect of Sitagliptin established in healthy albino rats. In the next part of this experiment, the effect of dose of Temsirolimus 6.75mg/kg per day for one week on the hypoglycemic activity of Sitagliptin was carried out in the same animals.

In the next part of this experiment, all the animals in a group I was treated with Temsirolimus at a dose 1.44 mg/kg. On the 7th day, 6 hrs after administration of Temsirolimus, the rats were fasted for 18hrs. On the 8th day, Temsirolimus at a dose 1.44 mg/kg was administered orally to all the animals in group I. 60 minutes later, Sitagliptin 9 mg/kg was administered to group I. Blood samples were collected thereafter at different time intervals at 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hours and were analyzed by GOD/POD method. Blood glucose levels were expressed as mg/dl of blood.

2.3. In diabetic rats

2.3.1. Experiment of diabetes mellitus

Diabetes was induced in the rats by STZ (50 mg kg-^{1}) intraperitonally into the 19h fasted rats. Blood samples were collected after 24h and blood glucose levels were considered as diabetic. The blood samples were collected after 24h and blood glucose levels were estimated. Albino rats which have shown more than 250 mg dL^{-1} blood glucose level were considered as diabetic. The blood glucose levels were monitored for further four days. From this it was confirmed that diabetes was induced in 48h and stabilized within 7 days. These animals were used for further studies. For the next phase study the same procedure was carried out as mentioned in the healthy rats above. Blood samples were collected subsequently at over-mentioned intervals, and glucose levels were estimated. The percentage blood glucose reductions at various time intervals were calculated.

3. Results

3.1. Effect of vehicle per se (2% w/v gum acacia suspension) administration and long term fasting on blood glucose levels in healthy albino rats

Acacia suspension (2% w/v) which has been used as vehicle for administration of the study drugs, by itself did not affect the blood glucose levels in healthy rats. Further the long term fasting (38 hrs) which inevitable occurs in these experiments was also doing not had any marked influence on the blood sugar levels in these animal species. The results of these findings are compiled in the Table 1 and graphically depicted in Fig.1.

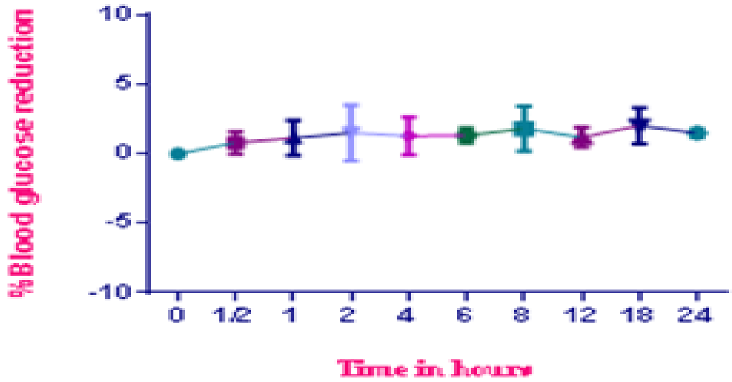

Fig. 1: Blood Glucose Level after the Administration of 2% Gum Acacia Suspension in Healthy Albino Rats.

Table 1: Blood Glucose Levels after the Administration of 2% Gum Acacia Suspension in Healthy Albino Rats

TIME	Blood Glucose Level (mg %)							Percentage Blood Glucose Reduction						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN±SEM
0	96	103	99	104	101	97	100.0±1.32	0	0	0	0	0	0	0
½	97	101	96	103	99	99	99.17±1.05	-1.04	1.9	3.03	.96	1.98	-2.06	0.7950±0.79
1	94	105	102	99	97	96	98.83±1.66	2.08	-1.9	-3.03	4.8	3.96	1.03	1.150±1.28
2	91	105	103	95	103	94	98.50±2.39	5.20	-1.9	-4	8.65	-1.98	3.09	1.503±2.01
4	93	102	97	101	99	102	99.00±1.44	3.125	.9	2.02	2.88	3.96	-5.15	1.289±1.36
6	95	103	98	100	100	96	98.17±1.56	1.04	0	1.01	3.84	.99	1.03	1.318±0.53
8	94	99	102	97	104	93	98.17±1.78	2.08	3.88	-3.03	6.73	-2.97	4.123	1.802±1.64
12	96	104	96	98	101	95	98.33±1.43	0	-.97	3.03	3.03	0	2.06	1.192±0.71
18	93	103	94	101	99	100	98.33±1.63	3.125	0	5.05	5.05	1.98	-3.09	2.019±1.29
24	94	100	98	103	100	96	98.5+1.31	2.08	2.91	1.01	.96	.99	1.03	1.497±0.33

3.2. Influence of temsirolimus on blood glucose levels in healthy albino rats

In the present study the per se effect of Temsirolimus (6.75 mg/kg) was assessed It is evident from the table.2 that, treatment of Temsirolimus (6.75 mg/kg) has no indicates that Temsirolimus does not possess any hypoglycemic effect the results of these findings are compiled in Table 2 and graphically depicted in Fig.2.

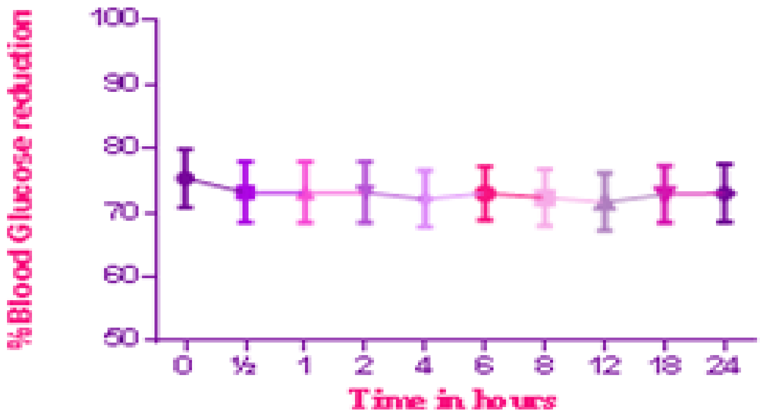

Fig. 2: Blood Glucose Level after the Administration of Temsirolimus in Healthy Rats.

Table 2: Blood Glucose Levels after the Administration of Temsirolimus in Healthy Albino Rat

TIME	Blood Glucose Levels(mg%)							Percentage Blood Glucose Reduction						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN±SEM
0	78	60	84	76	89	65	75.33±4. 1	-	-	-	-	-	-	-
½	75	57	82	74	88	63	73.17±4.1	3.8	5	2.38	2.63	1.12	3.07	3.007±.54
1	78	58	81	75	87	60	73.17±4.7	0	3.33	3.57	1.31	2.24	7.69	3.023±1.07
2	75	56	82	74	88	64	73.17±4.76	3.8	6.66	2.38	2.63	1.12	1.53	3.027±.82
4	72	57	83	76	83	62	72.17±4.0	7.6	5	1.19	0	6.74	4.61	4.206±1.24
6	73	58	80	78	85	64	73.00±4.7	6.4	3.33	4.76	-2.6	8.98	1.53	3.733±1.64
8	73	59	83	75	84	60	72.33±4.2	6.4	1.66	1.19	1.31	5.61	7.69	3.977±1.19
12	71	56	80	76	85	62	71.67±4.9	8.9	6.66	4.76	0	4.49	4.61	4.916±1.21
18	75	58	81	75	86	62	72.83±4.2	3.8	3.33	3.57	1.31	3.37	4.61	3.339±.44
24	74	57	82	74	87	64	73±4.531	5.1	5	2.38	2.63	2.24	1.53	3.153±0.62

3.3. Effect of temsirolimus pre-treatment on hypoglycemic effect of sitagliptin in healthy albino rats

Onset of hypoglycemia (the time taken to reduce blood glucose level to the extent of 15- 20%), duration of hypoglycemia (p<0.001) and peak hypoglycemia were the parameters considered for the evaluation of influence on sitagliptin induced hypoglycemia. Present study showed that. In healthy albino rats Pretreatment with Temsirolimus(6.75 mg/kg for seven days) has significantly altered the onset of hypoglycemia (15.50+1.470 to21.40±.5468), p< 0.001, at the 2nd hour, significantly enhanced the peak hypoglycemia (29.70+.6,035 %) before treatment to (44.47+1.214 %) after treatment(p<0.001) at 4th hour and duration of hypoglycemia was also significantly enhanced from about 12 hrs to more than 24 hrs (i.e. 18.71±.3883 to 20.43±. Present study showed that. induced by sitagliptin. The results of these findings are compiled in Tables 3, 4 and graphically depicted in Fig.3 and 4.

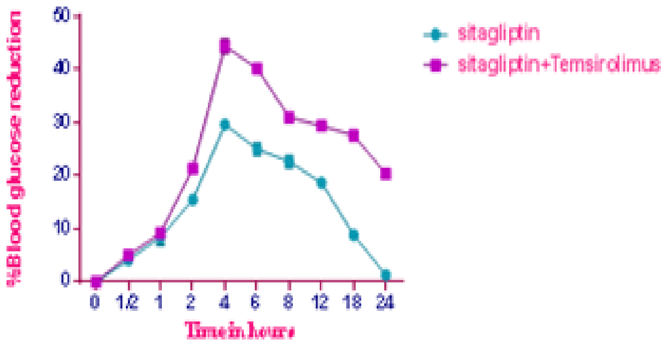

Fig. 3: Percentage Blood Glucose Reduction with Sitaglyptin in Healthy Albino Rats Before and After Temsirolimus Treatment.

Table 3: Blood Glucose Levels with Sitagliptin in Healthy Albino Rats Before and After Temsirolimus Treatment

TIME	Blood Glucose Levels with Sitagliptin							Blood Glucose Levels with Sitagliptin+Temsirolimus						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN ±SEM
0	98	98	103	96	99	99	98.83±.94	98	86	92	102	84	89	91.83±2.85
½	93	95	100	93	95	92	94.67±1.17	91	81	89	97	79	86	87.17±2.71
1	87	90	98	84	95	89	90.50±2.11	88	78	84	94	75	81	83.33±2.82
2	83	83	87	81	84	83	83.50±0.80	78	68	70	80	66	71	72.17±2.28
4	70	69	74	65	70	69	69.50±1.17	54	45	49	55	48	54	50.83±1.66
6	76	72	73	74	75	74	74.00±0.57	58	49	55	61	50	56	54.83±1.88
8	79	74	76	75	76	78	76.33±0.76	65	58	63	72	58	62	63.00±2.12
12	80	81	84	78	80	79	80.33±0.84	69	60	66	73	61	60	64.83±2.21
18	90	91	92	88	90	89	90.00±0.57	70	61	67	75	62	63	66.33±2.21
24	97	96	102	95	98	97	97.5±0.9916	74	68	75	82	69	71	73.17±2.088

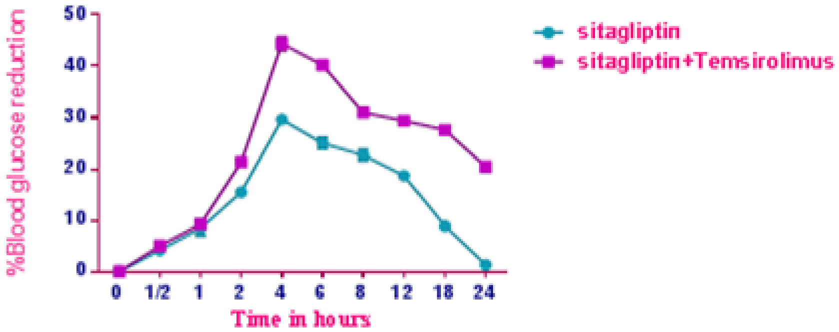

Fig. 4: Percentage Blood Glucose Reduction with Sitagliptin in Healthy Albino Rats Before and After Temsirolimus Treatment.

Table 4: Percentage Blood Glucose Levels with Sitagliptin in Healthy Albino Rats Before and After Temsirolimus Treatment

HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN±SEM
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
½	5.10	3.06	2.91	3.13	4.04	7	4.207±0.65	7.14	5.81	3.26	4.90	5.95	3.37	5.072±.62
1	11.22	8.16	4.85	12.5	4.04	10.10	8.478±1.4	10.20	9.30	8.69	7.84	10.71	8.98	9.287±.42
2	15.30	15.30	15.5	15.6	15.15	16.16	15.50±.14	20.40	20.9	23.91	21.56	21.42	20.22	21.40±0.54***
4	28.57	29.59	28.15	32.29	29.29	30.30	29.70±0.60	44.89	47.6	46.07	46.07	42.85	39332	44.47±1.21***
6	22.4	26.53	29.12	22.9	24.24	25.25	25.07±1.01	40.8	43.02	40.19	40.19	40.47	37.07	40.29±.77***
8	19.38	24.4	26.21	21.87	23.23	21.21	22.72±0.99	33.67	32.55	29.41	29.41	30.95	30.33	31.05±0.70***
12	18.36	17.34	18.44	18.75	19.19	20.20	18.71±0.38	29.59	30.23	28.43	28.43	27.38	32.58	29.44±0.74***
18	8.16	7.142	10.67	8.33	9.09	10.10	8.915±0.53	28.57	29.06	26.47	26.47	26.19	29.21	27.66±0.58***
24	1.02	2.04	0.97	1.04	1.01	2.02	1.350±0.21	24.40	20.93	19.60	19.60	17.85	20.22	20.43±0.89***

* Significant at $p< 0.05$; ** Highly significant at $p<0.01$; *** Very highly significant at $p<0.001$

* Represents the comparison of Sitagliptin with Sitagliptin+Temsirolimus interaction

3.4. Effect of vehicle per se (2% w/v gum acacia suspension) administration and long term fasting on blood glucose levels in albino rats treated with dexamethasone

Acacia suspension (2% w/v) which has been used as vehicle for administration of the study drugs, by itself did not affect the blood glucose levels in rats treated with dexamethasone. Further the long term fasting (38 hrs) which inevitable occurs in these experiments was also done not had any marked influence on the blood sugar levels in these animal species. The results of these findings are compiled in the Table 5 and graphically depicted in the Fig.5.

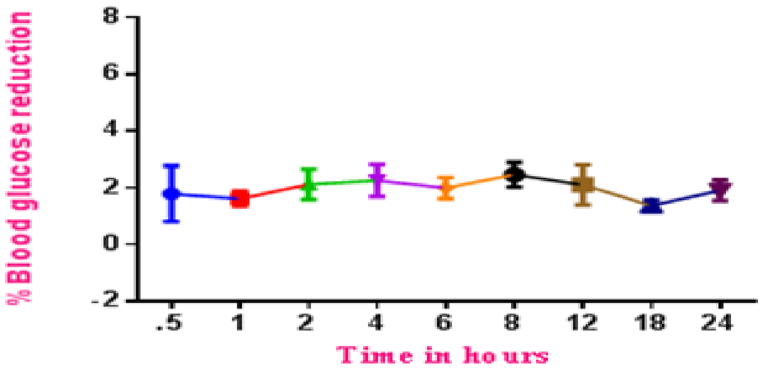

Fig. 5: Blood Glucose Level After the Administration of 2% Gum Acacia Suspension in Rats Treated with Dexamethasone.

Table 5: Blood Glucose Levels after the Administration of 2%Gum Acacia Suspension in Rats Treated with Dexamethasone

TIME	Blood Glucose Level (mg%)							Percentage Blood Glucose Reduction						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN±SEM
0	147	155	158	162	175	148	157.5±4.1	-	-	-	-	-	-	-
½	148	154	157	160	170	139	154.7±4.1	-0.68	0.64	0.63	1.23	2.85	6.08	1.792±0.97
1	143	153	156	160	173	145	155±4.45	2.72	1.29	1.26	1.23	1.14	2.02	1.610±0.25
2	145	151	152	162	171	144	154.2±4.6	1.36	2.58	3.79	0.00	2.28	2.70	2.118±0.53
4	144	150	151	160	174	145	154.0±4.2	2.04	3.22	4.43	1.23	0.57	2.02	2.252±0.56
6	145	152	152	159	172	146	154.3±4.8	1.36	1.90	3.79	1.85	1.71	1.35	1.993±0.37
8	143	151	156	156	173	143	153.7±4.4	2.72	2.58	1.26	3.70	1.14	3.37	2.462±0.43
12	140	154	157	157	170	147	154.2±4.5	4.76	0.64	0.63	3.08	2.85	0.67	2.105±0.70
18	145	153	155	159	173	147	155.3±4.1	1.36	1.29	1.89	1.85	1.14	0.67	1.367±0.18
24	144	150	156	161	171	145	154.5±4.3	2.04	3.22	1.26	0.61	2.28	2.02	1.907±0.36

3.5. In the present study the per se effect of temsirolimus (6.75 mg/kg) was assessed

It is evident from the table.6 that, treatment of Temsirolimus (6.75 mg/kg) has no Significant influence on the blood glucose levels in rats treated with dexamethasone. This Indicates that Temsirolimus does not possess any hypoglycemic effect. The results of these findings are compiled in Table 6 and graphically depicted in Fig.6.

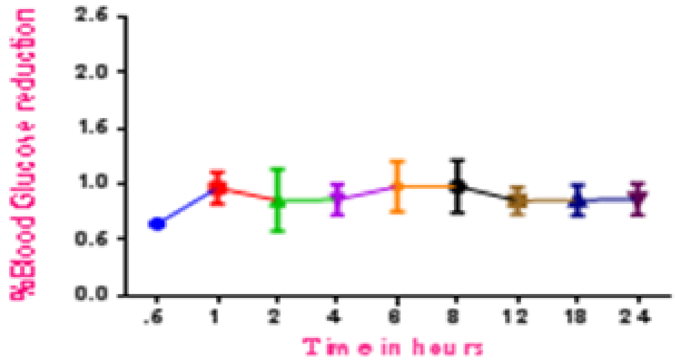

Fig. 6: Blood Glucose Levels after Administration of Temsirolimus in Rats Treated with Dexamethasone.

Table 6: Blood Glucose Levels after the Administration of Temsirolimus in Rats Treated with Dexamethasone

TIME	Blood Glucose Level (mg%)							Percentage Blood Glucose Reduction						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN±SEM
0	162.	148.	152	158	160.	145.	154.2±2.810	-	-	-	-	-	-	-
½	161.	147.	151.	157	159.	144.	153.2±2.810	0.610	0.675	0.657	0.630	0.625	0.680	0.6462±0.011
1	160.	147.	151	156.	159.	143.	152.7±2.789	1.230	0.670	0.657	1.260	0.625	1.370	0.9687±0.1436
2	161.	148.	149.	157.	158.	144.	152.8±2.750	0.617	0.000	1.970	0.630	1.250	0.680	0.8578±0.275
	160.	146.	151	157	159	144.	152.8±2.798	1.230	1.350	0.650	0.630	0.625	0.690	0.8625±0.136
6	160.	146.	150.	157	160	143.	152.7±3.007	1.230	1.350	1.300	0.632	0.000	1.370	0.9803±0.22620
8	161.	145.	151	157	159.	144.	152.8±2.971	0.617	2.027	1.300	0.630	0.625	0.680	0.9798±0.23590
12	160.	147.	151	157.	158.	144	152.8±2.651	1.230	0.675	0.657	0.630	1.250	0.680	0.8537±0.12240
18	161.	147.	150	157.	158.	144.	152.8±2.774	0.610	0.670	1.310	0.630	1.250	0.680	0.8583±0.134
24	160.	147.	151	157.	159.	143.	152.8±2.833	1.230	0.680	0.657	0.630	0.625	1.370	0.8653±0.13890

3.6. Effect of vehicle per se (2% w/v gum acacia suspension) administration and long term fasting on blood glucose levels in diabetic rats

Acacia suspension (2% w/v) which has been used as vehicle for administration of the study drugs, by itself did not affect the blood glucose levels in diabetic rats. Further the long term fasting (38 hrs.) which inevitable occurs in these experiments was also done not had any marked influence on the blood sugar levels in these animal species. The results of these findings are compiled in the Table 7 and graphically depicted in the Fig.7.

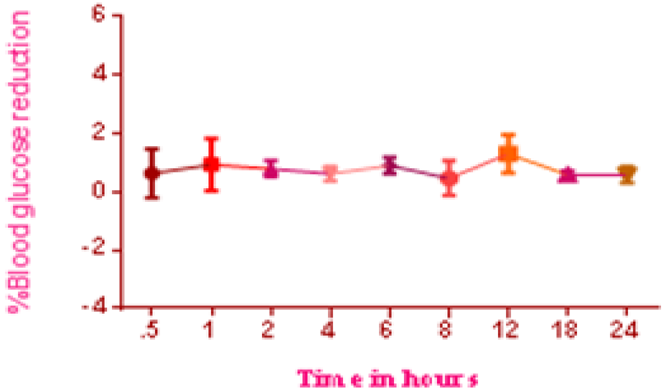

Fig. 7: Blood Glucose Levels after Administration of Gum Acacia Suspension in Diabetic Rats.

Table 7: Blood Glucose Levels after the Administration of 2%Gum Acacia Suspension in Diabetic Rats

TIME	Blood Glucose Level(mg%)							Percentage Blood Glucose Reduction						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN ±SEM
0	242	285	266	246	262	234	255.8±7.65	-	-	-	-	-	-	-
½	238	282	270	250	261	225	254.3±8.56	1.65	1.05	-1.5	-1.6	.38	3.84	0.6367±0.83
1	239	288	263	251	252	228	253.5±8.46	1.23	-1.05	1.12	-2.0	3.8	2.56	0.9383±0.88
2	241	285	262	245	260	230	253.8±7.93	.41	0	1.5	.4	.76	1.70	0.7950±0.27
4	241	280	266	245	262	233	254.5±7.24	.41	1.75	.75	.406	0	.42	.6227±0.24
6	241	284	262	245	257	232	253.5±7.54	.41	.35	1.5	.406	1.90	.854	.9033±0.26
8	240	284	265	251	260	228	254.7±8.03	.82	.35	.37	-2.0	.76	2.56	0.4717±0.60
12	244	280	261	244	261	225	252.5±7.74	-.82	1.75	1.87	.81	.38	3.84	1.305±0.64
18	241	284	263	245	261	232	254.3±7.66	.41	.35	1.12	.406	.38	.854	.5868±0.13
24	242	284	266	242	259	233	254.3±7.74	0	.35	-1.1	-2.7	2.67	-3.84	.5858±0.26

3.7. Effect of temsirolimus pre-treatment on hypoglycemic effect of sitagliptin in diabetic rats.

Onset of hypoglycemia (the time taken to reduce blood glucose level to the extent of 15- 20%), duration of hypoglycemia (the time duration in which more than 20% reduction in blood glucose level is maintained) and peak hypoglycemia were the parameters considered for the evaluation of influence on sitagliptin induced hypoglycemia. Present study showed that. Temsirolimus(6.75 mg/kg for seven days) has significantly altered the onset of hypoglycemia (19.44+.1993 to25.41±.7803), $p< 0.001$, at 2nd hour, significantly enhanced the peak hypoglycemia (41.54+.8438 %) before treatment to (50.71+.9011 %) after treatment($p<0.001$)at 4th hour and duration of hypoglycemia was also significantly enhanced from about 12 hrs to more than 24 hrs (i.e. 21.18±.7621 to 20.78±1.139, $p<0.001$) induced by sitagliptin. The results of these findings are compiled in Tables 8,9,10 and graphically depicted in Fig.8 and 9.

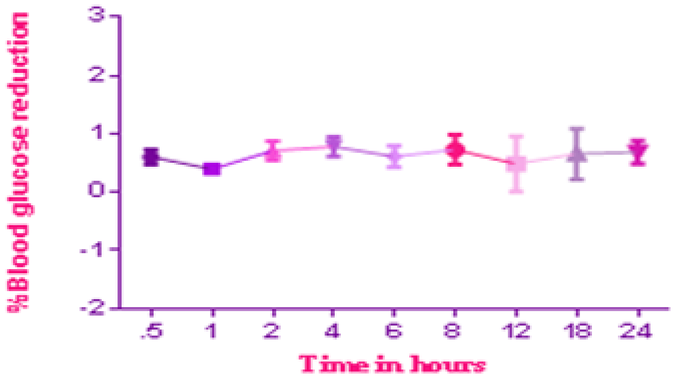

Fig. 8: Blood Glucose Level after the Administration of Temsirolimus in Diabetic Rats.

Table 8: Blood Glucose Levels after the Administration of Temsirolimus in Diabetic Rats

TIME	Blood glucose level(mg%)							Percentage blood glucose level reduction						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN ±SEM
0	282	286	346	290	300	302	301.0±9.54	-	-	-	-	-	-	-
½	281	283	343	289	298	301	299.2±.9.30	.35	1.04	.86	.344	.66	.33	.5973±.12
1	280	285	345	289	299	301	299.8±9.60	.709	.349	.28	.344	.33	.33	.3903±.064
2	280	283	342	288	298	300	298.5±9.20	.7	1.04	1.156	.68	.66	0	.7060±.16
4	279	282	343	288	299	301	298.7±9.50	1.06	1.39	.86	.68	.33	.33	.7750±.17
6	281	285	345	289	297	298	298±9.96	.35	.34	.28	.34	1	1.32	.6058±.18
8	279	287	345	287	296	299	298.8±9.688	1.06	-.34	.28	1.03	1.33	.99	0.7250±0.25
12	281	280	342	289	298	302	298.7±9.38	.35	2.09	1.156	-1.37	.66	0	0.4810±0.47
18	276	286	344	285	302	297	298.3±9.88	2.12	0	.57	1.72	-.66	.16	0.6517±0.43
24	280	282	345	288	299	299	298.8±9.81	.7	1.39	0	0.68	.33	.99	0.6817±0.19

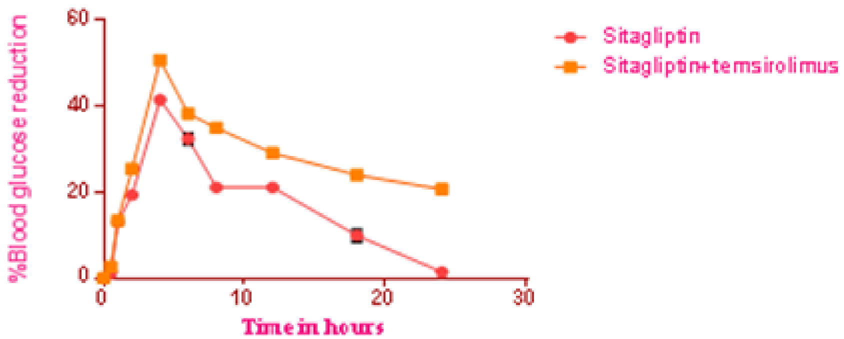

Fig. 9: Percentage Blood Glucose Reduction with Sitaglyptin in Diabetic Rats Before and after Temsiorlimus Treatment.

Table 9: Blood Glucose Levels with Sitagliptin in Diabetic Rats Before and after Temsirolimus Treatment

TIME	Blood Glucose Levels With Sitagliptin							Blood Glucose Levels With Sitagliptin+Temsirolimus						
HRS	H	B	T	HB	BT	TH	MEAN±SEM	H	B	T	HB	BT	TH	MEAN ±SEM
0	284	343	347	293	270	305	307.0±12.90	280	253	340	291	281	310	292.5±12.13
½	279	340	342	292	265	300	303.0±12.9	275	251	337	289	278	308	289.7±12.14
1	250	290	310	260	230	266	267.7±11.6 ±	245	220	304	254	243	268	255.7±11.60
2	230	276	280	237	215	246	247.3±10.5	208	180	261	220	212	230	218.5±10.92
4	170	205	209	172	148	175	179.8±9.45	135	129	178	145	130	149	144.3±7.491
6	175	240	240	198	184	210	207.8±11.2	170	152	205	175	165	185	175.3±7.424
8	196	255	245	203	195	224	219.7±10.5	180	170	225	189	181	205	191.7±8.196
12	220	280	275	223	215	240	242.2±11.7	205	185	242	198	193	220	207.2±8.483
18	260	310	320	275	230	265	276.7±13.6	212	193	265	211	218	235	222.3±10.16
24	278	341	339	292	267	298	302.5±12.6	219	210	278	219	222	242	231.7±10.23

Table 10: Percentage Blood Glucose Reduction with Sitagliptin in Diabetic Rats Before and after Temsirolimus Treatment

TIME	Percentage Blood Glucose Levels with Sitagliptin							Percentage Blood Glucose Levels with Sitagliptin+Tems						
HRS	H	B	T	HB	BT	TH	MEAN±SE	H	B	T	HB	BT	TH	MEAN±SEM
0	-	-	-	-	--	-	-	-	-	-	-	-	-	-
½	1.76	.87	1.4	.3	1.85	1.6	1.297±0.24	3.21	4	2.64	2.06	1.77	2.25	2.655±0.34
1	11.9	15.4	10.6	11.2	14.81	14.75	13.15±0.85	12.5	13.04	10.58	12.71	13.51	13.54	13.49±.15*
2	19.0	19.5	19.3	19.1	20.37	19.34	19.44±0.19	25.71	28.8	23.23	24.39	24.51	25.80	25.41±0.78***
4	40.1	40.2	39.7	41.2	45.18	42.62	41.54±0.84	51.78	49.01	47.64	50.17	53.71	51.93	50.71+0.90***
6	38.3	30.0	30.8	32.4	31.85	31.14	32.44±1.23	39.28	39.52	39.70	39.86	41.2	40.32	[illegible]*
8	30.9	25.6	29.3	30.7	27.77	26.55	28.51±.8 9	35.71	32.80	36.76	35.05	35.51	33.87	34.96±0.58***
12	22.5	18.3	20.7	23.8	20.37	21.31	21.18±0.76	26.78	26.87	28.82	31.95	31.3	29.03	29.13±0.88***
18	8.4	9.6	7.78	6.14	14.81	13.11	9.973±1. 35	24.28	23.71	22.05	27.49	22.4	24.19	24.02±0.79***
24	2.11	.58	2.3	.34	1.11	2.29	1.455±0.36	21.78	16.99	18.23	24.74	20.9	21.93	20.78±1.14***

* Significant at p<0.05; ** Highly significant at p<0.01; *** Very highly significant at p<0.001

* Represents the comparison of Sitagliptin with Sitagliptin+Temsirolimus interaction

4. Discussion

Diabetes is defined as a disorder exhibiting hyperglycemia caused by deficient insulin action, which is determined by both the capacity to secrete insulin from pancreatic Beta- cells and insulin action in peripheral insulin- sensitive tissue such as muscle and liver, requiring lifelong treatment. As per the research statistics, Hypertension is more prone to occur with diabetes. Hypertension also requires treatment for a prolonged period.If a patient is suffering from diabetes mellitus as well as hypertension, he has to use antidiabetic drug Sitaglptin and anticancereous agent like Temsorlimus. In such instances, there is a possibility of occurrence of drug interactions. Our pilot study has indicated that drug interactions occur when Temsirolimus and Sitaglptin administered concomitantly at therapeutic doses. However, the therapeutic dose was found to influence the antidiabetic effect significantly.

In case of diabetes mellitus regulation of blood glucose level is highly essential and important. But when a drug potentiates the effect of antidiabetic agent, the severe hypoglycaemia may be developed or if it inactivates the antidiabetic agents then the doses may be ineffective. There are several reports that Temsirolimus inhibit the isoenzymes of CYP-450 enzyme system. The isoenzymes that is affected by temsirolimus is CYP3A4. There is a possibility that drug- drug interaction may occur between Temsirolimus and the drugs metabolised by these enzymes.

in the present study DPP-4 inhibitor sitagliptin an oral antidiabetic agent and Temsirolimus an anticancer drug used in renal cell carcinoma is being used to understand, evaluate and confirm the drug-drug interaction between them. In this project interaction between the above mentioned classes of drugs were assessed in normoglycaemic rats, diabetic rats and Dexamethasone induced hyperglycemic rats.

In the first phase of study per se Effect of Temsirolimus on blood glucose level on healthy albino rats was evaluated. It was observed that Temsirolimus dose (6.75 mg/kg) has failed to influence the blood glucose indicating Temsirolimus does not possess any hypoglycaemic activity in albino rats indicating that the possible interactions with oral hypoglycaemic agents is not pharmacodynamic type In the second phase of this experimental influence of Sitagliptin (9mg/kg) was administered and onset of hypoglycaemia, extent of hypoglycaemia and duration of hypoglycaemia were assessed then to the same animals after 15 days, temsirolimus(6.75 mg/kg) were given for 7 days and then on the 8th day influence of this pre-treatment on the hypoglycemia produced by Sitagliptin was studied. It is evident from the findings of the results that in healthy rats, Pretreatment with Temsirolimus(6.75 mg/kg for seven days) has significantly altered the onset of hypoglycemia(15.50+1.470 to21.40±.5468), p< 0.001, at 2nd hour, significantly enhanced the peak hypoglycemia (29.70+.6035 %) before treatment to (44.47+1.214 %) after treatment,(p<0.001) at 4th hour and duration of hypoglycemia was also significantly enhanced from about 12 hrs to more than 24 hrs, (i.e 18.71±.3883 to 20.43±.8961, p<0.001) induced by Sitagliptin.

Our studies in rats and rabbits suggested that drug-drug interaction occurs between Temsirolimus and oral antidiabetic agent sitagliptin when they are used concomitantly in healthy conditions similarly drug-drug interaction occurs between Temsirolimus and Sitagliptin, when they are used concomitantly in dexamethasone induced rats. However the interaction in the pathophysiological conditions like in diabetes was not clear. Hence, in the fourth phase of our study the diabetic rats (streptozotocin induced diabetic rats) were used, sitagliptin was given to diabetic animals and the onset of hypoglycaemia duration of hypoglycemia and peak antidiabetic effect were determined To the same animals, Temsirolimus (6.75 mg/kg) pretreatment for one week as usual and again oral antidiabetic agents i.e.sitagliptin was given, Temsirolimus(6.75 mg/kg for seven days) has significantly altered the onset of hypoglycemia(19.44+.1993 to25.41±.7803), p< 0.001, at 2nd hour, significantly enhanced the peak hypoglycemia (41.54+.8438 %) before treatment to (50.71+.9011 %) after treatment (p<0.001) at 4th hour and duration of hypoglycemia was also significantly enhanced from about 12 hrs to more than 24 hrs, (i.e 21.18±.7621 to 20.78±1.139, p<0.001) induced by sitagliptin.Since the Temsirolimus has not shown significant effect on onset of hypoglycaemia, it may be inferred that Temsirolimus do not interefere with absorption of oral antidiabetic agents. However Temsirolimus have significantly enhanced the hypoglycemia in both induced by Sitagliptin. This may be due to fact that Temsirolimus mainly inhibit and CYP3A4, which is involved in the metabolism of sitagliptin. The above observations suggest that the interation between Temsirolimus and sitagliptin is very intense and it demands the readjutment of dose and frequency of oral antidiabetic agents when they are used concomitantly. The results of the whole study are summarized in Tables 11 and 12.

5. Conclusion

The present study concluded that, during simultaneous treatment of diabetes mellitus with hypertension, Temsirolimus do interact with Sitagliptin at therapeutic doses. Therefore it is necessary to adopt therapeutic drug monitoring so as to readjust dose and frequency of administration of these drugs, when they are used concominantly to avoid the patients from severe hypoglycaemia.

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