IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

STUDY OF PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTION OF HIMPLASIA WITH SITAGLIPTIN IN RATS

Dhanapuram Akhila Banu[1]*, Kallu golla dinesh[1],Gopi Mareedu[2], Vivek B[3], Velmurugan C[4]

1-Senior Research Scientist, Radiant Research Services Pvt Ltd., Bangalore, Karnataka-560058.
2-Head Technical Operatations, Radiant Research Services Pvt Ltd., Bangalore, Karnataka -560058.
3- Department of Pharmacology, T. John College of Pharmacy, Bangalore, Karnataka -560083
4- Department of Pharmacology, PGP College of Pharmaceutical Sciences and Research Institute, Namakkal, TamilNadu-637207.

Corresponding Author*

Dhanapuram Akhila Banu,

Senior Research Scientist,

Radiant Research Services Pvt Ltd.,

Bangalore,

Karnataka-560058.

ABSTRACT

Sitagliptin is given as an oral antidiabetic drug to treat Diabetes Mellitus. Himplasia may be co-prescribed along with sitagliptin to treat benign prostate hyperplasia. As such no information is available regarding the interaction taking place between sitagliptin and himplasia. Hence the present work has been aimed to find out the interaction with among the above said drugs in rodent model. Studies were conducted in normal and alloxan induced diabetic rats with oral doses of 9 mg / kg B.W of sitagliptin, and 54 mg /k g of himplasia and their combinations with adequate washout periods in between thetreatments. Blood samples were collected at regular time intervals in rats throughretro orbital puncture. All the blood samples were analyzed for blood glucose by GOD / POD method in pharmacodynamic studies and the serum sitagliptin concentrations were estimated by UV Spectrophotometry. Serum insulin was estimated by chemiluminescence assay. Sitagliptin showed hypoglycemic action in both normal and diabetic rats and the peak action was observed at 6 h. Hypoglycemia was observed with himplasia at 4th hour and the combination of sitagliptin and himplasia showed synergestic response in blood glucose levels. The serum sitagliptin concentrations were not altered by the co-administration

of drugs. A serum insulin level was potentiated by himplasia co-administered with sitagliptin. Thus it could be concluded that the co-prescription of himplasia should be taken for clinical benefits in diabetic patients. However, further studies should be carried out in non rodent species and in clinical settings to confirm the beneficial effect of Himplasia coprescription with Sitagliptin.

Keywords: Diabetes mellitus, Sitagliptin, Himplasia, Interactions, Insulin, Estimation

INTRODUCTION

The drugs acting at various receptors as agonist, partial agonists and antagonist produce a variety of pharmacodynamic actions in the tissue possessing those receptors. The mechanisms of such interaction at the receptor sites form the pharmacodynamic basis for the therapeutic use of such drugs. Some unexpected but interesting interaction were also found to occur in therapy involving similar mechanisms e.g. Several anabolic steroids have been reported to increase the activity of coumarin anticoagulants due to increased affinity of the anticoagulant for the receptor sites [1,2].

Dynamic that alter the concentration of plasma potassium level alter the therapeutic effect of cardiac glycosides, such as digoxin as well as other antiarrhythmic drugs. Angiotensin converting enzyme inhibitors have a potassium sparing effect, such that concurrent use of potassium supplements or potassium sparing diuretics may lead to dangerous hyperkalemia.

Some of the disorders like diabetes, hypertension and cardiac dysrrhythmias exist lifelong. Some disorders like diabetes may precipitate other disorders in the long run leading to existence of several disorders simultaneously. Some diseases like tuberculosis, leprosy are not easily cured because of the organism's resistance against the chemotherapy. Such situations (existence of simultaneous multiple disorders or resistant single disease) demand the use of more than one drug simultaneously known as polypharmacy or multidrug therapy.[3,4]

Himplasia significantly relieved the symptoms of BPH along with reduction in prostate size revealed by pelvic ultrasonography. Himplasia acts by dual mode of action which is by inhibition of 5-alpha reductase enzyme with alpha receptor blocking action. By this Himplasia controls the conversion of testosterone to dihydrotestosterone along with relaxation of prostatic smooth muscles[5]. 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 precipitate drug interactions at the receptor level leading to decreased effect of K^+ ATP channel openers and decreased / increased effect of Himplasia. [6] The drug treatment given to the patient is expected to improve the condition of the patient and should lead to further deterioration. Hence care should be taken to avoid the possibility for over medication / under medication / unwanted effects of the combinations particularly used in critical disorders. [7] It is desirable if more information is generated on the safety of such drug combinations by conducting more studies in this area.

MATERIALS AND METHODS

Male albino wistar rats weighing between 175-250 g were procured from registered breeders (Sri Venkateshwara 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-drug interaction studies.

Dose determination:

The usual human therapeutic dose (TD) of Sitagliptin is 100 mg and Himplasia 750 mg. The doses of 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 and 54 mg/kg B.W Himplasia. [8]

Preparation of drug solutions:

Sitagliptin phosphate (STP) solution: 18 mg of Sitagliptin was weighed accurately and dissolved in 2 ml of normal saline. Himplasia: 750 mg of drug was weighed accurately and dissolved in 20 ml of normal saline.

Studies on the influence of himplasia on the pharmacokinetics of sitagliptin in normal healthy rats

GROUPS	TREATMENT	NUMBER OF ANIMALS
Group-I	**Normal Control**	**6**
Group-II	**Himplasia (54 mg/Kg) + Sitagliptin (9 mg/Kg) (p.o)**	**6**

Stage I	All groups administered with vehicle control & blood samples were collected at different time intervals for serum glucose estimation.
Stage II	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: Himplasia (54 mg/kg B.W**)**
Stage IV	Group-II: Himplasia followed by Sitagliptin after 30 min

A washout period of 6 days was maintained in between all the stages. 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. Diabetes was induced in rats by the administration of Alloxan monohydrate in two doses, i.e. 100 mg and 50 mg/kg body wt (Intra peritoneal) *i.p* for two consecutive days. [9] Blood glucose levels were estimated using glucose oxidase-peroxidase method in Semi Auto Analyser. Serum Sitagliptin levels were estimated using UV-Spectrophotometry. 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}). Serum insulin levels were analysed by Chemiluminescence assay. [10-12]

STASTICAL ANALYSIS

One-way analysis of variance (ANOVA) followed by Dunnett's method of multiple comparisons was employed using Graph pad Instat 5.0 software.

RESULTS

STAGE-I: Effect of vehicle on blood glucose level in rats

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

Table: Blood glucose level in rats treated with vehicle

Time(h)	Group-I	Group-II
0	67.68±0.472	68.00±0.666
1	66.80±0.328	67.89±0.621
2	65.54±0.711	67.56±0.353
3	64.75±0.343	66.32±0.446
4	63.21±0.323	66.01±0.356
6	62.46±0.283	65.78±0.886
8	61.92±0.198	64.22±0.932
10	61.74±0.804	63.89±0.685
12	60.52±0.700	63.00±0.568

Table: Percent blood glucose change

Time(h)	Group-I	Group-III
0	-	-
1	-1.300±0.824	-0.16±0.089
2	-3.16±0.996	- 0.64±0.167
3	-4.329±0.588	-2.47±0.301
4	-6.60±0.460	-2.92±0.589
6	-7.712±0.957	-2.97±0.245
8	-8.510±0.638	-5.55±0.286
10	-8.776±0.675	-6.04±0.832
12	-10.57±0.1962	-7.35±0.456

STAGE-II: Effect of sitagliptin on blood glucose level in rats

Sitagliptin induced hypoglycaemia was studied by administering the TD of 9 mg/kg body weight to in the actual laboratory conditions. TD of sitagliptin produced - 59.573 ±1.464% percent blood glucose change at 6 hr. The result of effect of sitagliptin on blood glucose level in rats (Groups - II) was given in the tables.

Table: Effect of sitagliptin (9 mg/kg B.W) on blood glucose levelin rats.

Time(h)	Group-I	Group-II
0	66.98±0.992	65.95±1.409
1	66.00±0.788	58.06±1.940
2	65.74±0.811	46.61±1.174
3	64.65±0.443	39.61±0.521
4	63.41±0.923	30.29±0.717
6	63.06±0.683	26.56±0.657
8	62.02±0.298	38.31±0.466
10	61.94±0.904	44.36±0.968
12	60.32±0.790	57.36±1.533

Table: Percent blood glucose change

Time(h)	Group-I	Group-II
0	-	-
1	-1.463±0.664	-11.963±1.995
2	-1.851±0.755	-29.327±1.295
3	-3.478±0.768	-39.939±0.996
4	-5.329±0.442	-54.626±1.097
6	-5.852±0.223	-59.573±1.464
8	-7.405±0.465	-41.926±0.712
10	-7.524±0.742	-32.161±1.481
12	-10.07±0.960	-13.420±2.344

STAGE-III: Effect of himplasia (54 mg/kg B.W) on blood glucose level in rats

The result of the blood glucose levels and the percent blood glucose reduction with himplasia alone was tabulated in the table. TD of himplasia shows percent blood glucose change of -52.48 ±1.407 % was observed at 4 hr which was hypoglycaemic in group-II.

Table: Effect of himplasia (54 mg/kg B.W) on blood glucose level in rat

Time(h)	Group-I	Group-II
0	67.29±0.766	64.168±1.255
1	66.99±0.321	58.588±1.114
2	65.96±0.453	53.945±1.293
3	64.98±0.356	46.648±1.196
4	64.21±0.386	30.215±1.043
6	63.88±0.986	42.188±0.839
8	63.42±0.532	49.234±1.325
10	63.19±0.685	52.516±1.191
12	63.00±0.568	62.575±1.434

Table: Percent blood glucose change

Time(h)	Group-I	Group-III
0	-	-
1	-0.445±0.789	-8.697±1.503
2	-1.976±0.567	-15.921±1.743
3	-3.432±0.901	-27.306±1.613
4	-4.577±0.789	-52.489±1.407
6	-5.067±0.345	-34.258±1.131
8	-5.751±0.786	-23.623±1.787
10	-6.093±0.332	-18.191±1.605
12	-6.375±0.456	-2.842±1.934

STAGE-IV, Effect of himplasia (54 mg/kg B.W) by sitagliptin after 30 min on blood glucose level in rats

The results of the blood glucose levels and the percent blood glucose reduction with himplasia followed by sitagliptin after 30 min, was tabulated in the tables. It was observed that administering of himplasia followed by sitagliptin after 30 min produced response with a peak response of percent blood glucose change of - 70.537 ±1.0862% at 6 hr. Hypoglycaemia produced was observed by administering combination of himplasia and sitagliptin was

Table: Effect of himplasia (54 mg/kg B.W) followed by sitagliptin after 30 min on blood glucose level in rats.

Time(h)	Group-I	Group-III
0	69.59±0.980	67.908±0.766
1	68.86±0.461	56.345±1.518
2	68.05±0.278	46.106±1.187
3	67.94±0.745	35.855±0.991
4	66.95±0.889	27.113±0.744
6	66.37±0.336	20.023±0.589
8	65.23±0.587	27.071±0.782
10	64.00±0.451	33.653±0.997
12	63.98±0.395	43.646±0.847

Table: Percent blood glucose change

Time(h)	Group-I	Group-II
0	-	-
1	-1.049±0.724	-17.027±2.234
2	-2.212±0.906	-32.099±1.748
3	-2.376±0.508	-47.234±1.457
4	-3.793±0.479	-60.073±1.096
6	-4.627±0.420	-70.537±1.0862
8	-6.265±0.422	-60.135±1.152
10	-8.032±0.674	-50.440±1.469
12	-8.061±0.987	-35.726±1.247

The serum sitagliptin levels TD of sitagliptin

The serum sitagliptin levels before and after treatment with Himplasia, were presented in table shows no significant variation.

Table: Serum sitagliptin concentrations after oral administration of sitagliptin TD in normal rats

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	0.09	0.091	0.096	0.089	0.094	0.095	0.09±0.001
2	0.12	0.17	0.16	0.13	0.15	0.14	0.14±0.007
3	0.58	0.59	0.598	0.583	0.582	0.591	0.58±0.002

4	0.99	1.06	1.02	0.98	1.01	1.05	1.01±0.013
6	1.73	1.69	1.72	1.71	1.74	1.68	1.71±0.009
8	1.42	1.46	1.45	1.49	1.47	1.43	1.45±0.010
10	1.10	1.09	1.08	1.07	1.03	1.06	1.07±0.010
12	0.83	0.84	0.82	0.831	0.856	0.85	0.83±0.005

Table: Effect of Himplasia on the serum sitagliptin levels in normal rats

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	0.09	0.10	0.098	0.102	0.10	0.097	0.09±0.001
2	0.18	0.187	0.192	0.178	0.169	0.188	0.18±0.003
3	0.67	0.72	0.73	0.75	0.71	0.68	0.71±0.012
4	1.0	0.99	0.96	0.97	0.96	1.02	0.98±0.009
6	1.8	1.79	1.801	1.78	1.77	1.83	1.79±0.008
8	1.57	1.50	1.53	1.54	1.56	1.58	1.54±0.012
10	1.17	1.19	1.20	1.09	1.13	1.14	1.15±0.016
12	0.97	0.85	0.91	0.89	0.83	0.87	0.88±0.020

Effect of Himplasia on serum insulin levels by chemiluminescence assay:

Effect of sitagliptin on serum Insulin level:

The average blood glucose levels were decreased at 6^{th} hours. The average insulin levels peak at 6^{th} hours compared to 0^{th} &10^{th} hour.

Table: Serum insulin (µIU/ml) and blood glucose levels (mg/dl) before and after treatment with sitagliptin in normal rats (N=4)

Rats	**Body weight (gm)**	**Time (hr)**					
		0 hr		**1 hr**		**10 hr**	
		Blood Glucose (mg/dl)	**Serum Insulin**	**Blood Glucose (mg/dl)**	**Serum Insulin**	**Blood Glucose (mg/dl)**	**Serum Insulin**
R1	200	67.48	0.98	27.32	2.32	45.15	0.99
R2	200	67.32	0.83	26.44	2.59	44.89	0.97
R3	225	65.41	0.96	25.14	2.66	43.45	1.00

R4	150	68.59	0.89	28.97	2.84	46.82	0.93
Mean ± SEM		67.2±0.66	0.91± 0.03	26.96± 0.80	2.60± 0.10	45.07± 0.69	0.97± 0.15

Effect of Himplasia on serum insulin level:

The table shows the average blood glucose levels at 0, 1, 10 hr respectively when administered lonely and the average insulin levels at 0, 1, 10 hr respectively when administered along with sitagliptin.

Table: Serum insulin (µIU/ml) and blood glucose levels (mg/dl) before and after treatment with Himplasia in normal rats (N=4)

Rat	**Body weight (gm)**	**Time (hr)**					
		0 hr		**4 hr**		**10 hr**	
		Blood Glucose (mg/dl	**Serum Insulin**	**Blood Glucose (mg/dl)**	**Serum Insulin**	**Blood Glucose (mg/dl)**	**Serum Insulin**
R1	200	63.69	0.56	32.30	1.35	57.88	0.48
R2	225	68.58	0.78	33.42	1.76	55.69	0.62
R3	175	64.63	0.63	30.21	1.23	59.80	0.54
R4	175	66.34	0.82	31.56	1.62	61.02	0.68
Mean ±SEM		65.81± 1.07	0.69± 0.06	31.87± 0.67	1.49± 0.12	58.59± 1.16	0.58 ±0.12

Pharmacokinetic parameters

The below table shows pharmacokinetic parameters of Sitagliptin and himplasia, result reveal little variation but there is no significant variation in all parameters.

Table: Pharmacokinetic parameters of sitagliptin in normal rats

Kinetic Parameter	R_1	R_2	R_3	R_4	R_5	R_6	Mean ± SEM
AUC $_{0\text{-}12}$ (µg /ml/h)	11.60	11.76	11.70	11.64	11.67	11.59	11.66±0.02
AUMC $_{0\text{-}12}$ (µg /ml/h*h)	136.05	136.61	134.34	135.00	135.05	135.79	135.47±0.33
K_e (h^{-1})	0.0032	0.0031	0.0032	0.0033	0.0058	0.0029	0.00358±0.0004
AUC $_{0\text{-}\infty}$ (µg /ml/h)	17.78	17.83	17.45	17.33	18.00	18.12	17.75±0.125
AUMC $_{0\text{-}\infty}$ (µg /ml/h*h)	256.29	253.53	243.77	242.31	257.87	264.48	252.87±3.59
$T_{1/2}$ (h)	5.16	5.01	4.86	4.74	5.126	5.32	5.04±0.086

K_a (h^{-1})	0.768	0.768	0.768	0.768	0.768	0.768	0.768±0
Clearance (ml/h)	154.60	154.15	128.87	129.78	124.93	124.12	136.08±5.85
Clearance (ml/h/kg)	154.60	616.61	515.51	519.15	499.75	496.49	544.32±234.18
Vd_{ss} (ml)	2026.40	1990.24	1631.82	1645.23	1626.38	1649.48	1761.59±78.23
Vd_{ss} (ml/kg)	8105.60	7960.98	6527.30	6580.94	6505.55	6597.92	7046.38±312.9
Vd_{area} (ml)	1151.62	1115.46	904.399	889.12	924.18	954.44	989.87±46.53
Vd_{area} (ml/kg)	4604.49	4461.86	3617.754	3556.48	3696.74	3817.78	3959.48±186.1
MRT (h)	14.40	14.212	13.96	13.977	14.31	14.59	14.24±0.10
C_{max} (µg/ml)	1.73	1.69	1.72	1.71	1.74	1.68	1.71±0.009
T_{max} (h)	6	6	6	6	6	6	6.00±0.00

Table: Pharmacokinetic parameters of sitagliptin after treatment with TD of himplasia in normal rats

Kinetic Parameter	R_1	R_2	R_3	R_4	R_5	R_6	Mean ± SEM
AUC_{0-12} (µg /ml/h)	11.724	11.769	11.6345	11.6145	11.873	11.786	11.73±0.03
$AUMC_{0-12}$ (µg /ml/h*h)	137.48	139.53	134.75	132.61	134.09	138.04	136.42±0.91
K_e (h^{-1})	0.00252	0.00251	0.00289	0.00264	0.0030	0.0024	0.002±0.000009
$AUC_{0-\infty}$ (µg /ml/h)	19.92	19.91	18.31	18.56	18.07	20.09	19.06±1.22
$AUMC_{0-\infty}$ (µg /ml/h*h)	310.62	311.76	268.06	272.94	255.31	314.49	288.90±10.76
$T_{1/2}$ (h)	6.314	6.340	5.5112	5.669	5.238	6.393	5.911±0.204
K_a (h^{-1})	0.768	0.768	0.768	0.768	0.768	0.768	0.768±0.00
Clearance (ml/h)	112.92	112.99	122.84	121.17	124.50	111.94	117.72±2.33
Clearance (ml/h/kg)	451.69	451.98	491.39	484.69	498.03	447.79	470.93±9.32
Vd_{ss} (ml)	1613.49	1622.11	1638.11	1623.50	1597.07	1605.98	1616±5.901
Vd_{ss} (ml/kg)	6453.97	6488.45	6552.47	6494.01	6388.30	6423.99	6466.86±23.60
Vd_{area} (ml)	1028.98	1033.88	977.04	991.32	941.11	1032.83	1000.86±15.48
Vd_{area} (ml/kg)	4115.93	4135.54	3908.19	3965.28	3764.47	4131.34	4003.46±61.67
MRT (h)	15.589	15.65	14.635	14.699	14.128	15.647	15.06±1.39
C_{max} (µg /ml)	1.689	1.746	1.757	1.693	1.699	1.723	1.717±0.011
T_{max} (h)	6	6	6	6	6	6	6.00±0.00

DISCUSSION

The normal rat model served quickly to identify the interaction and diabetic rat model serve to validate the same response in actual condition of the drugs [13] 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 [14,15]. The himplasia produce hypoglycaemic effect when administered alone & enhanced the hypoglycaemic effect of sitagliptin in normal & diabetic rats when given in combination. It was also observed that himplasia did not alter the serum sitagliptin concentration, indicated that the interaction was pharmacodynamic interaction at receptor level might be due to its inhibitory action on alpha adrenoceptors as explained earlier. The therapeutic dose of himplasia was found to produce hypoglycaemia when administered alone in normal and diabetic rats. It potentiated the action of sitagliptin efficacy when administered along with that. It did not alter the pharmacokinetic parameters of sitagliptin in combination indicated that the interaction seen was pharmacodynamic interaction. The interaction occurred might be due to the effect of himplasia on the insulin release having inhibitory action on alpha-adrenoceptors. The results of the present study indicated that the therapeutic dose of himplasia enhanced the insulin secretion from basal level.

CONCLUSION

Himplasia produce hypoglycaemic effect in normal and diabetic rats when administered alone. It produce its effect in normal and diabetic rats and also potentiates the hypoglycaemic activity of sitagliptin when administered in combination. The pharmacokinetic parameter serum sitagliptin concentration was not altered with treatment of himplasia. The therapeutic dose of himplasia increased the insulin levels in normal rats. Himplasia appears to produce interaction with sitagliptin by pharmacodynamic mechanism i.e. by alpha adrenoceptor inhibition.

Since the interaction was found to produce hypoglycaemic effects was observed in rodent model, the interactions needs to be studied further in non-rodent models and also it can be extended in clinical studies.

REFERENCES

1. Longridge RGM, Gilman PMS, Barton GMG. Decreased anticoagulant tolerance with oxymethalone (Letter). Lancet 1971;2:90.
2. Edward MS & Curtis JR. Decreased anticoagulant tolerance with oxymetholone (Letter). Lancet 1971;2:221.
3. Mitchell JR, Arias L & Oates JA. Antagonism of the antihypertensive actions og guanethidine sulphate by desipramine hydrochloride. J Am Med Ass 1967;202:973.
4. Leishmann AWD, Mathews HL & Smith AJ. Antagonism of guanethidine by imipramine. Lancet 1963; 1:112.
5. Dr.Pranjal R.Modi and Dr.S.A.Kolhapure. Evaluation of clinical efficacy & safety of himplasia in BPH: A prospective, randomised, placebo – controlled, double blind, phase III clinical trial. Medicine update 2004; 12(6) :33-42.
6. 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.
7. Laurence DR, Bacharach AL. Evaluation of drug activities and pharmacometrics. London and New York: Academic press ;1964.
8. Manjula K.Pandit, John Burke, Antony B. Gustafson, Anil Minocha, Alan N. Peiris. Drug induced disorders of glucose tolerance.1993;118(7):529-39.
9. WHO Expert Committee on Diabetes Mellitus. Second Report. Geneva: WHO, 1980. Technical Report Series 646.

10. Sathish Kumar Konidala, Pampana V V Suresh Babu , Ranj, K S D Ranjitha. RP-HPLC Method Development and Validation for the Simultaneous Estimation of Sitagliptin and Simvastatin in Pharmaceutical Formulation. Asian J. Pharm. Ana. 2016; 6(2): 68-76.
11. P. Jaya Preethi. Herbal Medicine for Diabetes Mellitus: A Review. Asian J. Pharm. Res. 3(2): April- June 2013; Page 57-70
12. Lederballe Pedersen O. Long- term experiences with minoxidil in combination treatment of severe arterial hypertension. Acta Cardiol. 1997;32:283-93.
13. Brunchwaig A, Allen JG, Goldner MG, Gomori G. Alloxan. J Am med Ass 1943; 122:966.
14. Rakienten N, Rakienten ML,Nadakarni MV. Studies on the diabetogenic action of streptozocin. Cancer chemother Rep 1963;29: 91-92.
15. SM Bhanushali, KM Modh, IS Anand, CN Patel, JB Dave. Novel Approaches for Diabetes Mellitus: A Review. Research J. Pharmacology and Pharmacodynamics. 2010; 2(2):141-147.