A PKPD INTERACTION BETWEEN ORAL HYPOGLYCEMIC DRUG - SAXAGLIPTIN AND *TRIGONELLA FOENUM-GRAECUM* & HISTOPATHOLOGICAL STUDY

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ABSTRACT

Traditional medications obtained from the medicated herbal plants are used by about maximum percentage of world population for different chronic disease conditions. Diabetes (Hyperglycemia- high blood sugar level) is a very important metabolic disorder in different developed and developing countries including India. It causes very serious complications on health of human beings, especially in the rural and subrural areas. *Trigonella foenum-graecum* (Fenugreek plant) is a well-known traditionally used medicated herb, possesses different therapeutical activities. Fenugreek leaves have been used as traditional herbal medicines not only for hyperglycemia but also in hyperlipidimia, cellulitis and gastrointestinal disorders. Preliminary animal and human trials suggested the possible antihyperglycemic activity and antihyperlipidemic activity of oral fenugreek leaf extract. *T. foenum-graecum* leaves have also previously been shown to have antihyperglycemic and hypocholesterolemic effects on Type I and Type II Diabetes mellitus patients and experimental induced diabetic animals. However, the research so far on the hypoglycemic effect of fenugreek couldn't establish the optimum dose-level for experimental subjects. Hence, the research studies are required to study the pharmacodynamic and pharmacokinetic properties in order to determine the effect of fenugreek herb on the hyperglycemic patients who are taking the therapy with synthetic drugs. This study was taken up to discover the influence of *Trigonella foenum-graecum on* the pharmacokinetics and pharmacodynamics of Saxagliptin in rats. Results have proven the negative (decrease) effect of *Trigonella foenum-graecum on* pharmacokinetics but positive (increase) effect on pharmacodynamics of Saxagliptin.

Key words: Trigonella foenum-graecum, Saxagliptin, hypoglycemic effect.
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1. 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 [1-2].

Saxagliptin 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].Fenugreek (Scientific name-*Trigonella foenum graecum*) is the medicinal herb belongs to the family Leguminose. This is the common part of man's diet. These fenugreek green leaves and dried seeds are used for preparation of different food items at the same time it is used for medicinal use that is the old therapeutic practice of human's history of medical system. This is used to increase the flavor and colour of food items, and also modifies the quality of food. Fenugreek's seeds have therapuatic applications like antihypercholestremia, induce lactation, antimicrobial, gastric stimulant, for loss of appetite, antihyperglycemic action, galactogoguae, hepatoprotective action and antineoplasm. These medicinal applications on physiological actions including the antihyperglycemic and antihypercholestremic actions of fenugreek leaves and seeds are mainly attributable to the intrinsic dietary fiber constituents which has been promising the nutraceutical values[3-5].

There is scope for the potential herb-interactions between *Trigonella foenum graecum* and Sitagliptin. This can causefew 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.

2. MATERIALS AND METHODS

DRUGS AND CHEMICALS

Adult male wistar-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), distilled water *ad-libitum* for 14 days.These experimental protocols were in conducted according with IAEC/ CPCSEA.

EXTRACTION OF *T. FOENUM-GRAECUM* LEAVES:

Collection of Plant material

*T. foenum graecum'*s leaves were collected from the vegetable market of Hyderabad (Telangana). The healthy leaves were washed by using distilled water and the surface water drops were removed by using air drying. The fresh leaves are dried in hot-air oven at 40°C for 48 h and powered and are ready for the extraction process.

Procedure for Aqueous extraction

50 g of dried leaf powder of *T. foenum-graecum* is subjected to maceration with the 100 ml sterile distilled H_2O in the blender for 10 minutes. Then the resultant macerate was filtered through the double layered muslin cloth and centrifuged at 4000 rpm for 30 minutes. The supernatant was filter through the Whatmann filter paper No.1 and heat sterilized at 120°C per 30 minutes. The extract preparation was stored aseptically in the brown colored bottle at 4°C until future use.

Pretreatment

Albino rats were selected for this study (180-250gm), These animals are supplied by the NIN, Hyderabad, Telangana, animals are maintained under the suitable conditions in animal house. [IAEC number].The rats are kept in the animal cages and high fatty food and water are suppled 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 (SigmaAldrich; St. Louis; MO; USA) were injected intra-peritonially (freshly dissolve in the normal saline solution). After STZ administration, the animals are free accessed with food (pellet diet) & water. moderate polydipsia and marked polyuria are observed in diabetic hyperglycemic rats. After three days i.e. after 72hrs of injection, fasting blood glucose concentration were determined by following glucose levels by using commercial glucose estimation kitswith UV-Visible Spectrophotometer at 505nm based on the oxidase/peroxidase GOD/POD method. If any rats showing the fasting blood glucose level more than 150 mg/dL were consider the hyperglycaemic-rats and selected for the different grouping in the experimental design.

EXPERIMENTAL DESIGN:STUDY DESIGN OF GLIBENCLAMIDE- The hyperglycemic rats are divided in to 6 groups 6 animals in each.

Group I: Diabetic Control Group (0.5% Sodium.Carboxy Methyl Celluvose Suspension *Per Oral*)

Group II:*T. Foenum-Graecum* (100 Mg/Kg, *Per Oral*)

Group III:*T. Foenum-Graecum* (500 Mg/Kg. *Per Oral*)

Group IV: Combination Of Saxagliptin (2.5mg/Kg. *Per Oral*) + *T. Foenum-Graecum* (500 Mg/Kg *Per Oral*).

GroupV: Combination Of Saxagliptin (5 Mg/Kg. *Per Oral*) + *T. Foenum-Graecum* (500 Mg/Kg *Per Oral*).

Group VI: Saxagliptin (5 Mg/Kg. *Per Oral*) [6-11].

Pharmacokinetics study in hyperglycemic rat model:

Single dose Study

These pharmacokinetic studies are 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 dividing in to 6 different groups (n=6) and the follow the treatment was mention in the study design. Blood samples were collected at predetermined intervals of 0hr,1hr,2hr,4hr,8hr,12hr and 24hr in the hinto microcentrifugal tubes containing Na^+ citrate from retro-orbital pucture under di ethyl ether anaesthesia. The blood samples are subject to centrifugation at 3000rpm per 10minutes and plasma was stored at -20^{0C} for analysis and estimation of kinetic parameters as AUC 0 - ∞, Cmaxka, ke CL/F, Tmax, V/F, AUC 0-t &$t_{1/2}$.

Multiple dose study

The hyperglycemic rats are dividing into 6 different treatment groups same as mention in study design and daily treatment is carried for 21 days. Samples of blood are collected from different rat's groups on 0^{th},7^{th},14^{th},21^{st} day immediatly after drug treatment. Samples of blood are collected in to microcentrifugal tubes containing Na^{+}citrate from retro-orbital puncture under anaesthesia. These blood samples were subjected to centrifuged at 3000rpm per 10 minuts and plasma was stores at -20^{0} C for analysis and estimation of kinetic parameters as AUC 0 - ∞, V/F, ka, Cmax ,CL/F, Tmax,ke, AUC 0-t & $t_{1/2}$.

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 th interval of 0, 1, 2,4, 8, 12and 24hours by UV spectrophotometer.

Multiple dose study

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

Statistical Application

ANOVA followed by Dunnet test is performed for comparision between different groups of animals. *P* value fewer than 5% (P <0.05) was consider the statistically significant. All clinical data are expressed in the form of Mean±Sd. Pharmacokinetics data was calculated by using *pk* solversoftware and statistical analysis and graphical representations were done by *INSTANT graph pad* software.

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 resolution100_X.

3.RESULTS

Table1: Blood Glucose levels at 0^{th},1^{st},2^{nd},4^{th},8^{th}, 12^{th} and 24^{th}Hour after oral administration of *T. foenum-graecum,* Saxagliptin and combination of Saxagliptin + *T.foenum-graecum* in diabetic rats (n=6)

TIME (Hours)	TREATMENT (Single dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Saxagliptin (Dose in mg/kg)	Saxagliptin + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	500	5	2.5 + 500	5 + 500
	BLOOD GLUCOSE LEVEL (mg/dl)					
0	401.1±11.4	411.03±8.8	390.8±5.5	410.3±3.14	402.15±5.15	396.13±5.13
1	460.4±7.41	406.2±1.18**	361.7±8.2**	373.5±4.14**	371.14±11.1**	361.15±5.14**
2	462.8±5.14	342.1±11.3**	362.3±6.3**	361.11±1.24**	353.14±8.01**	351.14±6.12**
4	424.5±6.82	333.4±11.4**	363.2±3.1**	351.11±2.42**	351.14±6.13**	343.61±3.12**
8	422.1±7.4	297.51±2.2**	271.3±7.1**	261.21±3.3**	251.13±8.02**	241.15±5.12**

12	412.7±5.2	313.8±7.1**	293.4±6.1**	256.77±1.24**	247.19±6.12**	236.71±5.63**
24	413.8±9.1	328.4±4.6**	302.9±4.3**	261.51±4.12**	253.12±11.12**	236.14±9.12**

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)*
n - number of animals used.

Table 2:Blood Cholesterol levels at 0th,1st,2nd,4th,8th, 12th and 24thHour after oral administration of *T. foenum-graecum,* Saxagliptin and combination of Saxagliptin + *T. foenum-graecum*in diabetic rats (n=6)

TIME (Hours)	**TREATMENT (Single dose study)**					
	Diabetic Control	***T. foenum-graecum* (Dose in mg/kg)**		**Saxagliptin (Dose in mg/kg)**	**Saxagliptin + *T. foenum-graecum* (Dose in mg/kg)**	
	Vehicle	**100**	**500**	**5**	**2.5 + 500**	**5 + 500**
	BLOOD CHOLESTEROL LEVEL (mg/dl)					
0	198.3±11.3	204.4±8.1	202.13±11.3	208.13±10.22	195.16±10.21	195.2±10.81
1	200.1±11.3	200.4±8.4	194.11±13.2	192.53±3.21	185.16±5.18	186.5±9.13
2	201.14±5.31	183.3±3.9**	180.32±5.9**	176.13±.1.15**	174.33±.4.51**	172.14±4.33**
4	203.32±10.3	174.4±6.3**	170.42±6.3**	162.13±1.51**	153.21±4.73**	152.21±5.21**
8	202.9±4.14	146.1±6.5**	142.13±5.3**	140.13±10.53**	135.21±7.11**	130.12±10.14**
12	209.5±8.13	153.12±5.2**	150.42±7.1**	131.12±7.04**	130.16±6.51**	128.14±5.61**
24	211.2±6.6	177.04±7.3**	168.61±1.9**	144.24±10.15**	135.13±5.25**	133.16±10.21**

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)*
n - number of animals used.

Table 3: Blood Urea levels at 0th, 1st,2nd,4th,8th, 12th and24th Hour after oral administration of *T. foenum-graecum,* Saxagliptin and combination of Saxagliptin +*T. foenum-graecum*in diabetic rats (n=6)

TIME (Hours)	**TREATMENT (Single dose study)**			
	Diabetic Control	***T. foenum-graecum* (Dose in mg/kg)**	**Saxagliptin (Dose in**	**Saxagliptin + *T. foenum-graecum***

				mg/kg)	(Dose in mg/kg)	
	Vehicle	**100**	**500**	**5 + 500**	**2.5+500**	**5 + 500**
	BLOOD UREA LEVEL (mg/dl)					
0	199.4±11.5	204.6±8.3	202.15±11.5	209.14±10.22	196.16±10.24	195.4±10.81
1	200.2±11.3	200.4±8.2	194.13±13.4	192.53±3.22	185.14±5.14	186.4±9.13
2	201.13±5.31	183.2±3.4**	180.33±5.6**	176.13±.1.19**	174.33±.4.51**	171.15±4.35**
4	203.32±10.3	174.9±6.5**	170.43±6.2**	162.13±1.51**	154.23±4.72**	152.21±5.21**
8	202.5±6.13	146.2±6.3**	144.11±5.3**	140.12±10.53**	135.23±7.12**	130.12±10.14**
12	209.9±8.18	153.15±5.3**	150.43±7.3**	131.15±7.04**	130.19±6.52**	128.18±5.63**
24	211.4±6.5	177.06±7.3**	168.64±1.8**	144.25±10.18**	135.16±5.21**	133.18±10.24**

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)*
n - number of animals used.

Table 4: Blood Glucose levels at 0th,7th, 14th and 21st day after oral administration of *T. foenum-graecum*, Saxagliptin and combination of Saxagliptin and *T. foenum-graecum*in diabetic rats (n=6)

TIME (Day)	**TREATMENT (Multiple dose study)**					
	Diabetic Control	***T. foenum-graecum* (Dose in mg/kg)**		**Saxagliptin (Dose in mg/kg)**	**Saxagliptin + *T. foenum-graecum* (Dose in mg/kg)**	
	Vehicle	**100**	**500**	**5**	**2.5 + 500**	**5 + 500**
	BLOOD GLUCOSE LEVEL (mg/dl)					
0	408.13±3.92	414.28±1.3	393.12±1.3	402.11±5.61	384.53±7.12	
7	393.18±5.4	237.23±1.5**	230.14±1.3**	215.64±7.99**	211.15±4.61**	202.19±6.13**
14	384.18±3.13	181.33±1.4**	154.13±2.3**	144.62±8.03**	136.15±5.63**	128.04±8.06**
21	390.35±1.4	130.15±2.3**	121.64±1.8**	116.16±5.91**	111.83±4.71**	102.24±7.04**

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)*
n - number of animals used.

Table 5: Blood Cholesterol levels at 0th,7th, 14th and 21st day after oral administration of *T. foenum-graecum,* Saxagliptin and combination of Saxagliptin and *T. foenum-graecum*in diabetic rats (n=6)

TIME (Day)	TREATMENT (Multiple dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Saxagliptin (Dose in mg/kg)	Saxagliptin + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	5	2.5 + 500	5	0.6 + 500
	BLOOD CHOLESTEROL LEVEL (mg/dl)					
0	192.24±10.9	187.43±5.5	181.16±11.3	177.16±8.13	170.13±7.93	168.05±7.13
7	192.81±9.3	104.16±8.3**	101.15±7.2**	101.53±5.72**	93.25±5.3**	90.12±4.61**
14	185.73±8.42	85.25±8.91*	83.74±8.3**	75.05±7.14**	71.53±4.41**	64.64±8.14**
21	190.4±6.54	72.36±9.5**	71.65±8.1**	65.89±8.04**	59.85±7.62**	54.84±4.96**

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)*
n - number of animals used.

Table 6: Blood Urea levels at 0th,7th, 14th and 21st day after oral administration of *T. foenum-graecum,* Saxagliptin and combination of Saxagliptin and *T. foenum-graecum*in diabetic rats (n=6)

TIME (Day)	TREATMENT (Multiple dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Saxagliptin (Dose in mg/kg)	Saxagliptin + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	500	5	2.5 + 500	5 + 500
	BLOOD UREA LEVEL (mg/dl)					
0	70.16±3.73	67.14±7.13	68.29±4.24	65.18±7.83	60.14±2.83	59.19±4.24
7	75.49±8.35	41.18±1.74**	37.41±5.09**	34.64±4.31**	31.45±1.16**	30.24±5.06**
14	78.54±8.32	33.19±6.83**	33.08±7.05**	28.08±3.81**	25.42±7.01**	20.03±8.61**
21	81.04±5.21	31.69±7.24**	31.48±7.54**	25.15±5.08**	20.48±5.02**	18.09±2.05**

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)*
n - number of animals used.

Table 7: Mean plasma Saxagliptin concentrations (µg/ml) (Single dose study)

Time (Hours)	Diabetic Control	Saxagliptin 5 mg/kg	Saxagliptin + *T. foenum-graecum*	
			2.5mg/kg+500mg/kg	5mg/kg+ 500mg/kg
1	0	2.72±0.03	2.66±0.06	2.60±0.03
2	0	5.60±0.04	4.98±0.04	5.10±0.07
4	0	5.20±0.03	4.60±0.02	4.99±0.06
8	0	4.19±0.03	3.58±0.04	4.08±0.04
12	0	3.19±0.02	2.81±0.05	3.04±0.04
24	0	2.08±0.01	1.68±0.04	1.86±0.03

Table 8: Effect of *T. foenum-graecum* on Pharmacokinetic parameters of Single dose administration of Saxagliptin in diabetic rats (n=6)

Pharmacokinetic parameter	Unit for Pharmacokinetic parameter	Saxagliptin 5mg/kg	Saxagliptin + *T. foenum-graecum*	
			2.5 mg/kg + 500 mg/kg	5mg/kg + 500 mg/kg
ka	**h^{-1}**	0.7440±0.021	0.6125±0.015*	0.7034±0.072
ke	**h^{-1}**	0.8916±0.103	0.9121±0.26	0.9262±0.433
$t_{1/2}$	**h**	3.05±0.04	3.03±0.03	3.04±0.08
V/F	**(mg/kg)/(µg/ml)**	1.58±0.03	1.68±0.05	1.72±0.09**
CL/F	**(mg/kg)/(µg/ml)/h**	0.07±0.04	0.08±0.08*	1.00±0.04*
Tmax	**h**	2.04±0.08	2.05±0.04	2.09±0.03*
Cmax	**µg/ml**	5.39±0.04	4.88±0.03**	5.19±0.08**
AUC 0-t	**µg/ml*h**	80.52±0.71	70.05±0.31**	76.04±0.43**
AUC 0 - ∞	**µg/ml*h**	100.71±0.52	81.06±0.44**	90.23±0.61**

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)*
n - number of animals used.

Table 9: Mean plasma Saxagliptin concentrations (µg/ml) (Multiple dose study)

Time (Days)	Diabetic Control	Saxagliptin 5 mg/kg	Saxagliptin + *T. foenum-graecum*	
			2.5 mg/kg + 500 mg/kg	5mg/kg + 500 mg/kg
1	0	2.53±0.04	2.34±0.04	2.35±0.03
7	0	6.05±0.04	4.61±0.02	5.41±0.02
14	0	5.00±0.02	4.24±0.09	4.55±0.04
21	0	4.48±0.03	3.40±0.02	3.71±0.02

Table 10: Effect of *T. foenum-graecum* on Pharmacokinetic parameters of Multiple dose administration of Saxagliptin in diabetic rats (n=6)

Pharmacokinetic parameter	Unit for Pharmacokinetic	Saxagliptin 5 mg/kg	Saxagliptin + *T. foenum-graecum*	
			2.5 mg/kg + 500	5mg/kg + 500

	parameter		mg/kg	mg/kg
ka	**h^{-1}**	0.050±0.063	0.045±0.017	0.047±0.033
ke	**h^{-1}**	0.040±0.02	0.032±0.01	0.043±0.04
$t_{1/2}$	**h**	3±0.03	3.02±0.03	3.03±0.06
V/F	**(mg/kg)/(µg/ml)**	1.60±0.03	1.38±0.02**	1.58±0.08*
CL/F	**(mg/kg)/(µg/ml)/h**	0.08±0.02	0.09±0.03	1.00±0.07
Tmax	**h**	2.02±0.06	2.07±0.06	2.14±0.04**
Cmax	**µg/ml**	6.10±0.04	4.78±0.03**	5.50±0.04**
AUC 0-t	**µg/ml*h**	92.34±0.72	65.16±0.22**	74.18±0.03**
AUC 0 - ∞	**µg/ml*h**	103.26±0.19	85.16±0.63**	94.81±0.68**

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)*
n - number of animals used.

3. DISCUSSION:

Pharmacodynamic study:

The combination of high dose of Saxagliptin (5 mg/kg) with 500mg/kg *T. foenum-graecum* showed maximum hypoglycemic action, decrease in serum cholesterol and urea levels. The effect produced by combination of Saxagliptin (2.5 mg/kg) with *T. foenum-graecum* was greater than the hypoglycaemic action produced by *T. foenum-graecum* (500 mg/kg) alone and Saxagliptin (5 mg/kg).

Pharmacokinetic study:

The Single dose study shows that, 96.33% decrease in AUC(0 - ∞) in 500mg/kg of *T. foenum-graecum* and 2.5mg/kg of Saxagliptin, 97.41% decrease AUC (0 - ∞) in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin when compared with the 5mg/kg of Saxagliptin group.

C max was decreased by 91.09% in 500mg/kg of *Trigonella foenum-graecum* and 2.5mg/kg of Saxagliptin, 99.03% in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin in single dose study when compared with the 5mg/kg of Saxagliptin group.

Significant decrease in absorption rate constant Ka by about 60.53% in Lower dose of 500mg/kg of *T. foenum-graecum* and 50mg/kg of Saxagliptin, 81.34% in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin when compared with the 5mg/kg of Saxagliptin group.

Significantly increase in clearance 2.19% in 500mg/kg of *T. foenum-graecum* and 2.5mg/kg of Saxagliptin. 5.45% in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin compared to 5mg/kg Saxagliptin when compared with the 5mg/kg of Saxagliptin group.

The multiple dose study shows that, 96.71% decrease in AUC(0 - ∞) in 500mg/kg of *T. foenum-graecum* and 2.5mg/kg of Saxagliptin. 98.21% decrease AUC(0 - ∞) in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin when compared with the 5mg/kg of Saxagliptin group.

C max was decreased by 88.36% in 500mg/kg of *T. foenum-graecum* and 2.5mg/kg of Saxagliptin, 96.61% in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin in multiple dose study when compared with the 5mg/kg of Saxagliptin group.

Significant decrease in absorption rate constant Ka by about 58.31% in Lower dose of 500mg/kg of *T. foenum-graecum* and 2.5mg/kg of

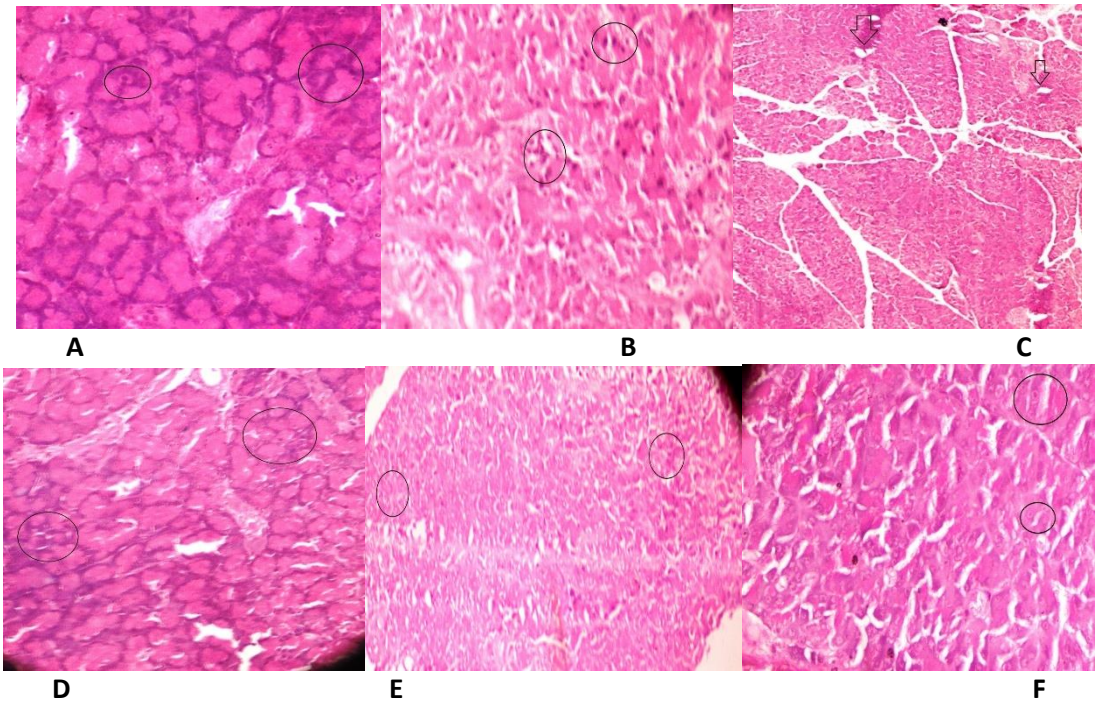
Saxagliptin, 76.41% in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin when compared with the 5mg/kg of Saxagliptin group.

Significantly increase in clearance 4.51% in 500mg/kg of *T. foenum-graecum* and 2.5mg/kg of Saxagliptin. 10.25% in 500mg/kg of *T. foenum-graecum* and 5mg/kg of Saxagliptin compared to 5mg/kg Saxagliptin group.

The exact reason behind the reduction in pharmacokinetic parameters was unknown but, it was understood that the combination of Saxagliptin extract with Saxagliptin 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 (Saxagliptin + *T. foenum-graecum*) involved in the increase the number of islets and recovered the partially damaged B cells in pancreas when compare to the Individual treatment.



H&S Staining of Pancreatic islets of Diabetic Control, *T. foenum-graecum*alone Saxagliptin alone and combination of *T. foenum-graecum*& Saxagliptin treated Diabetic Rats. 6A. diabetic control , 6B. 100mg of *T. foenum-graecum*6C. 500mg *T. foenum-graecum,* 6D.5mg of Saxagliptin.6E.500mg of *T. foenum-graecum*. +2.5mg of Saxagliptin, 6F. 500mg of *T. foenum-graecum*+5mg of Saxagliptin.

Slide A shows that pancreatic cells were damaged due to development of diabetes from STZ. Figure 6B shows that few pancreatic cells were damaged due to Saxagliptin. Figures 6C,6D,6E, 6F shows that B cells are regenerated in pancreatic tissue.

Normal ß-cells were observed in low and high doses of Saxagliptin and *T. foenum-graecum*. (Slides: 6D&6F). In the Saxagliptin

group more damaged ß-cells as compared with the 500mg of *T. foenum-graecum* +5mg of Saxagliptin and 500mg of *T. foenum-graecum* +2.5mg of Saxagliptin (Figures: 6B,6C&6E).

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, *T. foenum-graecum* was decrease the absorption and increase the clearance of Saxagliptin. Hence care must be taken when the combination is taken by diabetic patients.

5. CONCLUSION

The interaction appears to be pharmacokinetic interaction at absorption, elimination. *T. foenum-graecum*inhibits the absorption of Saxagliptin that results in a significant decrease in the bioavailability of the later and combination group with a lower dose of Saxagliptin and increment to the volume of islets in pancreas is observed in combination group when compare to individual treatment. Since the interaction was seen in rats it is likely to occur in humans leading to decreased activity of Saxagliptin that can need dose adjustments. Hence care must be taken when the combination is taken by the diabetic patients. The present study warrants next plan to find out the relevance of the interaction in human beings.

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