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In-Vivo Evaluation Of Drug-Herb Interaction Between Glibenclamide And *Trigonella Foenum-Graecum* By Pharmacodynamic, Pharmacokinetic And Histopathological Studies In Diabetes Mellitus Induced Rats

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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 Glibenclamide in rats. Results have proven the negative (decrease) effect of Trigonella foenum-graecum on pharmacokinetics but positive (increase) effect on pharmacodynamics of Glibenclamide.

Key words: *Trigonella foenum-graecum,* Glibenclamide, hypoglycemic effect

1. INTRODUCTION

Diabetic Mellitus (Hyperglycemia) is an endocrine disease and is 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].

Glibenclamide is a oral hypoglycemic drug, interferes with glucose transport modulation by ATP-sensitive potassium channels in peripheral tissues and NO-mediated vasorelaxation induced by a high glucose level. Glibenclamide interferes with mitochondrial bioenergetics in nonpancreatic cells by inducing the changes in the membrane ion permeability. Noninsulin dependent glucose transport via GLUT1 protein appears to be one of the possible additional mechanisms of the drug antidiabetic action[6].

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[7-10].

There is scope for the potential herb-interactions between *T. foenum graecum* and Glibenclamide. Glibenclamide can cause Thrombocytopenia, Cholestatic jaundice as a result, it precipitates potentially life-threatening effects. 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.[11-15]

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 present study was taken up to discover the influence of *T. foenum-graecum on* the pharmacokinetics and pharmacodynamics of Glibenclamide in diabetes induced rats.

2. MATERIALS AND METHODS

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 were 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 filtered 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[16].

DRUGS AND CHEMICALS

The oral hypoglycemic drug, 5 grams of Glibenclamide Pure drug- Powder dosage form (doses are 0.5 and 0.6 mg/kg) was manufactured by Alka Pharmaceutical Company Hyderabad, India. (supplied as a Gift sample).

The diabetogenic compound, 1gram of Streptozotocin Pure drug (dose is 60 mg/kg) Powder dosage form was manufactured by Sigma-Aldrich, St. Louis, MO, USA. (supplied as a Gift sample).

EXPERIMENTAL ANIMALS

Adult male Wistar-rats weighing between 150±20 g (Mahaveer Enterprises, Hyderabad, Telangana) were used in this experimental study. These animals were acclimatized to the standard laboratory conditions of suitable temperature (27°C±1°C) and maintained on 12:12 hours light and dark cycle in animal house. They were maintained in elevated rat's wire cages and provided with regular standard diet (Standard pellets - carbohydrates: proteins: fat in 42:18:40) and distilled water was given *ad-libitum* for 14 days. These experimental protocols were conducted in accordance with the guidelines of CPCSEA.

PRETREATMENT

The experimental rats were kept in the animal cages and high fatty food and water was supplied in the form of *ad libitum* for 15 days.

INDUCTION OF HYPERGLYCEMIA IN RATS BY STREPTOZOTOCIN (STZ) (60 mg/kg):

After 15 days of feeding with highly fatty food the rats were fasted for a period of 18 h before the induction of hyperglycemia and single dose administration of 60 mg/kg of Streptozocin (Sigma Aldrich, St. Louis, MO, USA) were injected intraperitonially (freshly dissolved in the normal saline solution). After STZ administration, the animals were freely accessed with food (pellet diet) and water. Moderate polydipsia and marked polyuria are observed in diabetic hyperglycemic rats. After three days i.e., after 72 h of injection, fasting blood glucose concentration were determined by following glucose levels by using commercial glucose estimation kits and reading at UV-Visible Spectrophotometer at 505 nm based on the Glucose oxidase/peroxidase (GOD/POD) method. The rats showing the fasting blood glucose level more than 150 mg/dl were considered as the hyperglycemic rats and selected for the different experimental groups of the study.

STUDY DESIGN:

The hyperglycemic rats were divided in to 6 groups with 6 animals in each group.

Group I: Diabetic Control Group (0.5% Sodium Carboxy Methyl Cellulose 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 Glibenclamide (0.5 mg/kg, *Per Oral*) + *T. foenum-graecum* (500 mg/kg, *Per Oral*)
Group V: Combination of Glibenclamide (0.6 mg/kg, *Per Oral*) + *T. foenum-graecum* (500 mg/kg, *Per Oral*).
Group VI: Glibenclamide (0.6 mg/kg, *Per Oral*) [17].

PHARMACODYNAMIC STUDY IN THE HYPERGLYCAEMIC EXPERIMENTAL RATS:

Single dose study

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

Multiple dose study

In this study, daily treatment was given to all groups of animals for 3 weeks as per the experimental design. Pharmacodynamic parameters like urea, cholesterol and glucose levels were estimated at the time interval of 0^{th}, 7^{th}, 14^{th} and 21^{st} day using UV-Visual spectrophotometer.

PHARMACOKINETIC STUDY IN HYPERGLYCEMIC EXPERIMENTAL RATS:

Single dose Study

These pharmacokinetic studies were carried out in hyperglycemic rats. These animals were housed in cages with free access to the diet and water *ad-libitum.* The overnight fasting rats were dividing into 6 different groups (n=6) and the treatment was followed as mentioned in the study design. Blood samples were collected at predetermined intervals of 0 h, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h in the microcentrifugal tubes containing Sodium citrate by retro-orbital puncture under diethyl ether anaesthesia. The blood samples were subjected to centrifugation at 3000 rpm per 10 minutes and plasma was stored at -20°C for analysis and estimation of kinetic parameters such as AUC 0-∞ (area under the curve 0-∞), ka (absorption rate constant), ke (elimination rate constant), CL/F (clearance/fraction absorbed), Cmax (maximum concentration of the drug achieved in the plasma), Tmax (time at which Cmax is attained), Tmax, V/F (Plasma volume/fraction absorbed), AUC 0-t and $t_{1/2}$.

Multiple dose study

The hyperglycemic rats were divided into 6 different treatment groups same as mentioned in study design and daily treatment was carried for 21 days. Samples of blood were collected from different experimental groups on 0^{th}, 7^{th}, 14^{th}, 21^{st} day immediately after drug treatment. Samples of blood were collected into microcentrifugal tubes containing Sodium citrate by retro-orbital puncture under anaesthesia. These blood samples were subjected to centrifugation at 3000 rpm per 10 minutes and plasma was stored at -20°C for analysis and estimation of kinetic parameters such as AUC 0-∞, ka, ke, CL/F, Cmax, Tmax, Tmax, V/F, AUC 0-t and $t_{1/2}$.

HISTOPATHOLOGICAL STUDY:

After estimation of last blood glucose level, the animals were sacrificed and histopathological study was carried out to estimate the inflammation and necrosis related changes in pancreas. The pancreatic tissues were stained using Haematoxylin and Eosin (H & E) stains and observed under the magnification of 1000x.

STATISTICAL APPLICATION:

ANOVA followed by Dunnet's test was performed for comparison between different groups of animals with significant differences. *P*-value less than 0.05 ($P < 0.05$) was considered as statistically significant. All the clinical data was expressed in the form of Mean±SD. Pharmacokinetic data was calculated by using *pk* solver software and statistical analysis and graphical representations were done by *INSTANT* Graph Pad software.

3. RESULTS

PHARMACODYNAMIC STUDY:

The combination of high dose of Glibenclamide (0.6 mg/kg) with 500 mg/kg *T. foenum-graecum* showed maximum hypoglycaemic activity along with decreased serum cholesterol and urea levels. The effect shown by combination of Glibenclamide (0.5 mg/kg) with *T. foenum-graecum* was found to be greater than the hypoglycemic effect showed by *T. foenum-graecum* (500 mg/kg) alone and Glibenclamide (0.6 mg/kg) alone according to the Table 1, 2, 3, 4, 5 and 6.

PHARMACOKINETIC STUDY:

The single dose study shows that there were drug concentrations increases up to 2nd hour after drug administration then it decreases continuously up to 24hours in all treated groups according to Table No.7.

The Single dose study shows that there was 19% decrease in the Area Under the Curve AUC(0-∞) at 500 mg/kg of *T. foenum-graecum* plus 0.5 mg/kg of Glibenclamide group and the 9.1% decrease in the AUC(0-∞) at 500 mg/kg of *T. foenum-graecum* plus 0.6 mg/kg dose of Glibenclamide group was observed when compared with 0.6 mg of Glibenclamide group according to the Table 8.

In the Single dose study, 14.8% decrease in Cmax (highest concentration of a drug in the blood after a dose given) at 500 mg/kg of *T. foenum-graecum* plus 0.5 mg/kg of Glibenclamide group and 10.02% decrease in Cmax at 500 mg/kg of *T. foenum-graecum* plus 0.6 mg/kg of Glibenclamide group were observed when compared with 0.6 mg of Glibenclamide group according to the Table 8.

In the Single dose study, 26% decrease in absorption rate constant Ka at the dose of 500 mg/kg of *T. foenum-graecum* plus lower dose (0.5 mg/kg) of Glibenclamide group and the 11% decrease in absorption rate constant Ka at the dose of 500 mg/kg of *T. foenum-graecum* plus high dose (0.6 mg/kg) of Glibenclamide were observed when compared with 0.6 mg of Glibenclamide group according to the Table 8.

In the Single dose study, 15% increase in Clearance (CL) at 500 mg/kg of *T. foenum-graecum* plus 0.5 mg/kg of Glibenclamide group and 31% increase in CL at 500 mg/kg of *T. foenum-graecum* plus 0.6 mg/kg of Glibenclamide were observed when compared with 0.6 mg of Glibenclamide group according to the Table 8.

The Multiple dose study shows that there were drug concentrations increases up to 7th day after drug administration then it decreases continuously up to 21 days in all treated groups according to Table 9.

The Multiple dose study shows that, 18.92% decrease in Area Under the Curve AUC(0 - ∞) at 500 mg/kg of *T. foenum-graecum* plus 0.5 mg/kg of Glibenclamide group and 10.26% decrease in

AUC(0-∞) at 500 mg/kg of *T. foenum-graecum* plus 0.6 mg/kg of Glibenclamide group were observed when compared with 0.6 mg of Glibenclamide group according to the Table 10.

In the Multiple dose study, 23.91% decrease in Cmax at 500 mg/kg of *T. foenum-graecum* plus 0.5 mg/kg of Glibenclamide and 11.6% decreases in Cmax at 500 mg/kg of *T. foenum-graecum* plus 0.6 mg/kg of Glibenclamide group were observed when compared with 0.6 mg of Glibenclamide group according to the Table 10.

In the Multiple dose study, 28.16% decrease in absorption rate constant Ka at the dose of 500 mg/kg of *T. foenum-graecum* plus lower dose (0.5 mg/kg) of Glibenclamide and the 11.43% decrease in absorption rate constant Ka at the dose of 500 mg/kg of *T. foenum-graecum* plus high dose (0.6 mg/kg) of Glibenclamide group were observed when compared with 0.6 mg of Glibenclamide group according to the Table 10.

In the Multiple dose study, 17.83% increase in Clearance (CL) at the dose of 500 mg/kg of *T. foenum-graecum* plus 0.5 mg/kg of Glibenclamide and the 28.09% increase in Clearance (CL) at the dose of 500 mg/kg of *T. foenum-graecum* plus 0.6 mg/kg of Glibenclamide compared to high dose Glibenclamide group were observed when compared with 0.6mg of Glibenclamide group according to the Table 10.

The exact reason behind the reduction in pharmacokinetic parameters was unknown but it was understood that the combination of *T. foenum-graecum* leaf extract with Glibenclamide in fact reduces the exposure of the synergic drugs without reducing the pharmacodynamic activity. Thus the proposed combination allows a safe therapy with less adverse effects.

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

TIME (Hours)	TREATMENT (Single dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Glibenclamide (Dose in mg/kg)	Glibenclamide + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	500	0.6	0.5 + 500	0.6 + 500
	BLOOD GLUCOSE LEVEL (mg/dl)					
0	402.1±12.4	412.0±9.8	391.8±6.5	411.3±4.14	401.15±6.15	397.13±6.13
1	461.4±8.41	407.2±1.19**	362.7±8.1**	374.5±5.14**	370.14±10.1**	360.15±6.14**
2	463.8±8.14	348.1±11.2**	366.3±6.1**	360.11±1.24**	356.14±8.02**	350.14±8.14**
4	428.5±6.81	335.4±11.9**	364.2±3.5**	350.11±2.45**	350.14±6.14**	347.61±3.19**
8	421.1±7.3	297.5±2.6**	272.3±7.2**	262.21±3.9**	250.13±8.03**	240.15±5.13**
12	413.7±5.1	313.9±7.3**	293.6±6.3**	258.77±1.21**	248.19±8.12**	239.71±5.61**
24	414.8±9.4	329.4±4.9**	303.9±4.5**	261.52±4.14**	254.12±11.14**	236.16±9.19**

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 24th Hour after oral administration of *T. foenum-graecum,* Glibenclamide and combination of Glibenclamide + *T. foenum-graecum* in diabetic rats (n=6)

TIME (Hours)	TREATMENT (Single dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Glibenclamide (Dose in mg/kg)	Glibenclamide + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	500	0.6	0.5 + 500	0.6 + 500
	BLOOD CHOLESTEROL LEVEL (mg/dl)					
0	198.4±11.4	204.5±8.2	202.14±11.4	208.14±10.23	196.18±10.23	195.30±10.83
1	200.3±11.2	200.6±8.4	194.12±13.3	192.51±3.23	185.18±5.18	186.90±9.14
2	201.1±5.32	183.5±3.9**	180.31±5.9**	176.14±.1.18**	174.31±.4.52**	172.15±4.36**

4	203.3±10.5	174.8±6.9**	170.41±6.5**	162.15±1.52**	154.21±4.71**	152.23±5.23**
8	202.9±6.14	146.3±6.5**	144.13±5.4**	140.15±10.55**	135.24±7.14**	130.13±10.15**
12	209.8±8.16	153.1±5.1**	150.46±7.3**	131.14±7.06**	130.18±6.53**	128.16±5.62**
24	211.5±6.9	177.1±7.1**	168.62±1.9**	144.21±10.19**	135.14±5.28**	133.19±10.22**

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 and 24th Hour after oral administration of *T. foenum-graecum*, Glibenclamide and combination of Glibenclamide + *T. foenum-graecum* in diabetic rats (n=6)

TIME (Hours)	**TREATMENT (Single dose study)**					
	Diabetic Control	***T. foenum-graecum* (Dose in mg/kg)**		**Glibenclamide (Dose in mg/kg)**	**Glibenclamide + *T. foenum-graecum* (Dose in mg/kg)**	
	Vehicle	**100**	**500**	**0.6**	**0.5 + 500**	**0.6 + 500**
	BLOOD UREA LEVEL (mg/dl)					
0	63.71±6.4	63.17±5.4	74.12±4.4	75.18±3.17	67.89±6.14	65.84±5.85
1	65.16±2.5	64.71±7.13	67.15±5.23	66.09±4.12	61.01±6.14	59.15±6.25
2	66.31±9.34	62.14±5.19	65.23±7.01	63.21±4.64	58.16±6.51	56.62±5.32
4	67.91±4.15	58.42±7.14	56.21±4.11*	52.14±5.61**	50.15±5.15**	48.07±5.16**
8	67.05±3.36	51.74±5.13**	46.6±4.15**	45.32±6.14**	44.06±4.11**	40.21±5.14**
12	68.11±1.14	51.0±5.21**	51.17±5.98**	49.15±3.81**	46.92±4.55**	44.45±5.11**
24	68.22±7.18	60.42±4.37	58.16±8.16	52.42±5.73**	49.17±4.3**	47.62±5.81**

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*,Glibenclamide and combination of Glibenclamide and *T. foenum-graecum* in diabetic rats (n=6)

TIME (Day)	TREATMENT (Multiple dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Glibenclamide (Dose in mg/kg)	Glibenclamide + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	500	0.6	0.5 + 500	0.6 + 500
	BLOOD GLUCOSE LEVEL (mg/dl)					
0	409.11±3.93	418.29±1.4	394.18±1.5	402.13±5.62	384.55±7.14	190.21±5.38
7	393.15±5.3	237.22±1.5**	230.19±1.3**	215.62±7.99**	211.13±4.63**	202.18±6.12**
14	384.15±3.15	181.32±1.9**	154.11±2.5**	144.61±8.05**	136.12±5.61**	128.01±8.04**
21	390.34±1.8	130.14±2.6**	121.63±1.8**	115.16±5.93**	111.82±4.73**	102.25±7.06**

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,* Glibenclamide and combination of Glibenclamide and *T. foenum-graecum* in diabetic rats (n=6)

TIME (Day)	TREATMENT (Multiple dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Glibenclamide (Dose in mg/kg)	Glibenclamide + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	500	0.6	0.5 + 500	0.6 + 500
	BLOOD CHOLESTEROL LEVEL (mg/dl)					
0	192.21±10.5	187.41±5.5	181.19±11.2	177.14±8.15	170.12±7.91	168.09±7.15
7	192.82±9.7	104.14±8.5**	101.16±7.3**	101.52±5.71**	93.27±5.4**	90.13±4.62**
14	185.71±8.41	85.24±8.92*	83.76±8.1**	75.04±7.14**	71.54±4.42**	64.61±8.11**
21	190.40±6.52	72.36±9.3**	71.64±8.1**	65.88±8.04**	59.84±7.62**	54.82±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,* Glibenclamide and combination of Glibenclamide and *T. foenum-graecum* in diabetic rats (n=6)

TIME (Day)	TREATMENT (Multiple dose study)					
	Diabetic Control	*T. foenum-graecum* (Dose in mg/kg)		Glibenclamide (Dose in mg/kg)	Glibenclamide + *T. foenum-graecum* (Dose in mg/kg)	
	Vehicle	100	500	0.6	0.5 + 500	0.6 + 500
	BLOOD UREA LEVEL (mg/dl)					
0	70.18±3.71	67.11±7.14	68.26±4.22	65.19±7.81	60.11±2.81	59.18±4.28
7	75.44±8.34	41.15±1.71**	37.42±5.09**	34.65±4.33**	31.44±1.18**	30.25±5.08**
14	78.51±8.31	33.18±6.81**	33.06±7.04**	28.06±3.82**	25.41±7.04**	20.04±8.63**
21	81.03±5.24	31.68±7.21**	31.46±7.51**	25.14±5.08**	20.44±5.02**	18.04±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 Glibenclamide concentrations (µg/ml) during Single dose study

Time (Hours)	Diabetic Control	Glibenclamide 0.6 mg/kg	Glibenclamide + *T. foenum-graecum*	
			0.5 mg/kg+500 mg/kg	0.6 mg/kg+ 500 mg/kg
1	0	2.63±0.03	2.59±0.06	2.51±0.03
2	0	5.51±0.04	4.89±0.04	5.01±0.07
4	0	5.11±0.03	4.51±0.02	4.93±0.06
8	0	4.13±0.03	3.51±0.04	4.03±0.04
12	0	3.15±0.02	2.88±0.05	3.09±0.04
24	0	2.03±0.01	1.65±0.04	1.85±0.03

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

Pharmacokinetic parameter	Unit for Pharmacokinetic parameter	Glibenclamide 0.6 mg/kg	Glibenclamide + *T. foenum-graecum*	
			0.5 mg/kg + 500 mg/kg	0.6 mg/kg + 500 mg/kg
ka	h^{-1}	0.75±0.088	0.64±0.019*	0.71±0.072

			(↓26%)	**(↓11%)**
ke	**h^{-1}**	0.88±0.106	0.91±0.26	0.92±0.451
$t_{1/2}$	**h**	10.05±0.04	10.03±0.03	10.04±0.08
V/F	**(mg/kg)/(µg/ml)**	1.59±0.03	1.69±0.05	1.71±0.09**
CL/F	**(mg/kg)/(µg/ml)/h**	0.08±0.04	0.09±0.08* **(↑15%)**	1.00±0.04* **(↑31%)**
Tmax	**h**	2.05±0.08	2.06±0.04	2.11±0.03*
Cmax	**µg/ml**	5.41±0.04	4.89±0.03** **(↓14.8%)**	5.16±0.08** **(↓10.02%)**
AUC 0-t	**µg/ml*h**	81.35±0.72	71.05±0.33**	77.04±0.41**
AUC 0 - ∞	**µg/ml*h**	101.76±0.53	81.09±0.49** **(↓19%)**	90.25±0.63** **(↓9.1%)**

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 Glibenclamide concentrations (µg/ml) during Multiple dose study

Time (Days)	Diabetic Control	Glibenclamide 0.6 mg/kg	Glibenclamide + *T. foenum-graecum*	
			0.5 mg/kg + 500 mg/kg	0.6 mg/kg + 500 mg/kg
1	0	2.59±0.04	2.39±0.04	2.36±0.03
7	0	6.09±0.04	4.65±0.02	5.44±0.02
14	0	5.01±0.02	4.26±0.09	4.58±0.04
21	0	4.49±0.03	3.41±0.02	3.72±0.02

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

Pharmacokinetic parameter	Unit for Pharmacokinetic parameter	Glibenclamide 0.6 mg/kg	Glibenclamide + *T. foenum-graecum*	
			0.5 mg/kg + 500 mg/kg	0.6 mg/kg + 500 mg/kg
ka	**h^{-1}**	0.05±0.064	0.05±0.018 **(↓28.16%)**	0.05±0.034 **(↓11.43%)**
ke	**h^{-1}**	0.04±0.03	0.03±0.02	0.04±0.06
$t_{1/2}$	**h**	10.00±0.03	10.02±0.03	10.03±0.06
V/F	**(mg/kg)/(µg/ml)**	1.61±0.03	1.39±0.02**	1.59±0.08*
CL/F	**(mg/kg)/(µg/ml)/h**	0.08±0.03	0.09±0.04 **(↑17.83%)**	1.00±0.08 **(↑28.09%)**
Tmax	**h**	2.03±0.06	2.08±0.06	2.15±0.04**
Cmax	**µg/ml**	6.11±0.04	4.79±0.03** **(↓23.91%)**	5.51±0.04** **(↓11.6%)**
AUC 0-t	**µg/ml*h**	92.35±0.72	65.17±0.22**	74.19±0.03**
AUC 0 - ∞	**µg/ml*h**	103.28±0.19	85.18±0.63** **(↓18.92%)**	94.82±0.69** **(↓10.26%)**

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.

HISTOLOGICAL STUDY

The histological study shows that combination therapy (Glibenclamide and *T. foenum-graecum*) involved in the increase of the number of islets and recovered the partially damaged B-cells in pancreas when compared to the individual treatment.

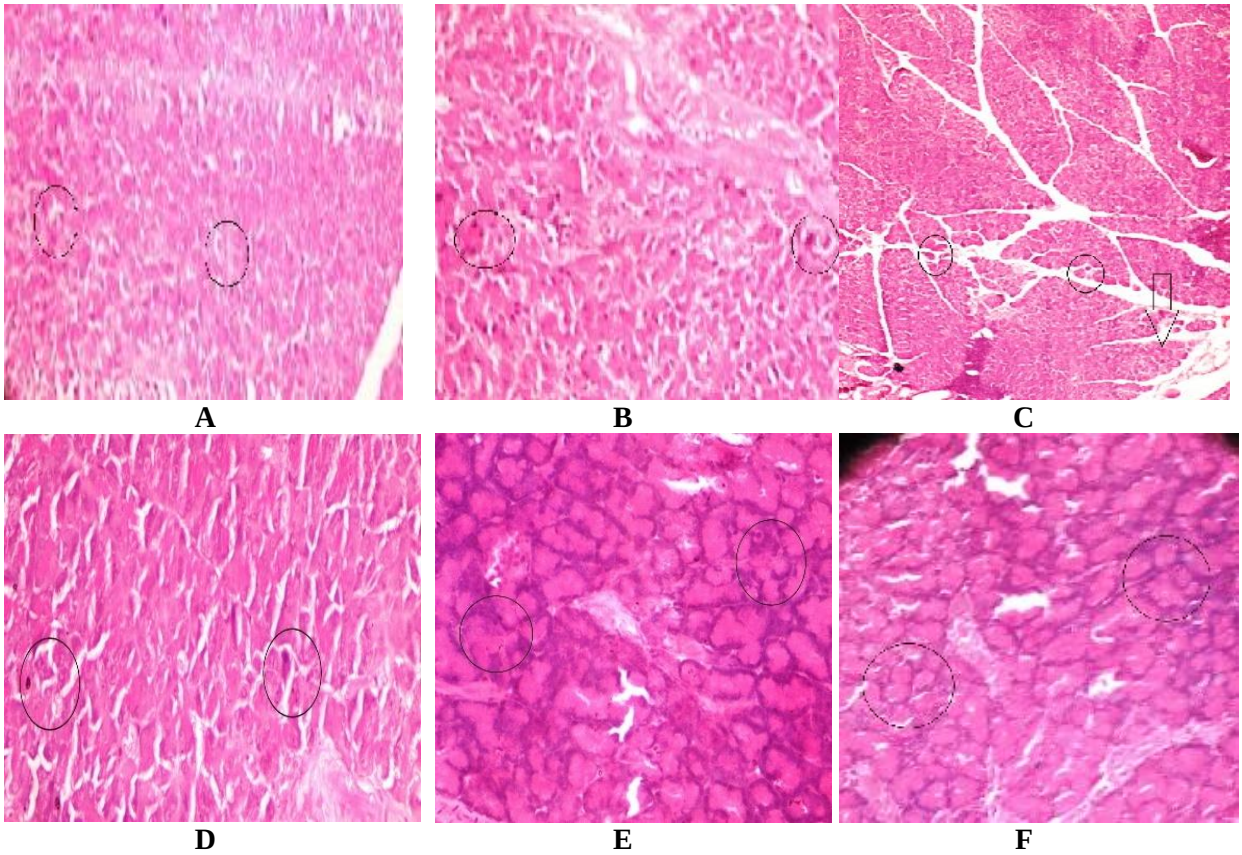

Figure 1: H& E Staining of Pancreatic islets of Diabetic Control, *Trigonella foenum-graecum* alone, Glibenclamide alone and combination of *Trigonella foenum-graecum*& Glibenclamide treated Diabetic Rats. (A. Diabetic control, B.100mg of *Trigonella foenum-graecum*, C. 500mg of *Trigonella foenum-graecum*, D. 0.6 mg of Glibenclamide, E. 500 mg of *Trigonella foenum-graecum* +0.5 mg of Glibenclamide, F. 500 mg of *Trigonella foenum-graecum* + 0.6 mg of Glibenclamide)

Slide A shows that pancreatic tissue was damaged due to administration of STZ, Slide B was shows that few number of B-cells are damaged due to Glibenclamide, but regenerative activity produced by *T. foenum-graecum* that was clear from Slides C, D, E and F.

Normal β-cells were observed in low and high doses of Glibenclamide and *T. foenum-graecum* (Slides E & F). In the Glibenclamide group more damaged β-cells as compared with the 500 mg of *T. foenum-graecum* + 0.6 mg of Glibenclamide and 500 mg of *T. foenum-graecum* + 0.6 mg of Glibenclamide (Slide D).

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 Glibenclamide 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 Glibenclamide produced increment to the volume of islets in pancreas compared to individual treatment.

It was observed from the results that combination of *T. foenum-graecum* and Glibenclamide increase the volume of Pancreatic islets compared to the individual therapy i.e., *T. foenum-graecum* alone and Glibenclamide alone.

Hence, the present study has revealed that *T. foenum-graecum* has decreased the absorption and increase the clearance of Pioglitazone. Hence care must be taken when the combination therapy is given to diabetic patients.

4. DISCUSSION

The use of herbs along with modern medicine is more common in the world. The patients are not aware of the complications related to such combination. The medicines are combined with the sole belief that it would provide exact benefit which may be true in some cases and such combinations may produce adverse effects in others. Hence, studying such interactions will definitely help in rationalizing such combinations for clinical use. Furthermore, some of the physicians are also not aware of such combinations and literature provided by studies such as the present study will help physician in choosing right doses and right combinations. Diabetes is a chronic disease requiring life-long treatment once it occurs. Hence, studying interactions of drugs used in diabetes will be more important than studying other drug interactions.

Nivitabishekam *et al.* had reported interaction of rosiglitazone with *Momordica charantia*, interaction of soybean with Carbamazepine and Omeprazole, interaction of garlic with different antihypertensive drugs and interaction of betel quid with calcium channel blockers earlier[18] which finally achieved the enhanced therapeutic effect with minimal adverse effects. Similarly our study reported the interaction of *T. foenum-graecum* and Glibenclamide which improved the volume of pancreatic islets compared to individual therapy i.e., *T. foenum-graecum* alone and Glibenclamide alone.

The interaction of modern medicine with herbs is a developing area with research activities being carried out in few areas in the world. The interaction of herbs with various classes of drugs have been reported and some drugs such as terfenadine and astemizole from the market due to such interactions. Fenugreek use in patients taking sulfonylureas may further decrease the blood glucose levels[19]. *Randazzo-moura* shown effect on the pharmacological action of glibenclamide in normal rats[20]. Greater hypoglycemic activity was noted in streptozotocin-induced diabetic rats receiving the combination of garlic extract (500 mg/kg) and glibenclamide (0.25 or 0.5 mg/ kg) than either of the drug given alone[21]. Concomitant administration of ginger extract (25 or 50 mg/kg) and glibenclamide (5 mg/kg) in streptozotocin (STZ)-induced diabetic rats, decreased the non-fasting blood glucose level significantly. The blood glucose levels should be monitored in patients taking sulfonylureas and ginger together, to avoid the occurrence of hypoglycemia[22]. Carvedilol will interact with pharmacokinetics and Pharmacodynamic of glipizide in normal and diabetic rats[23].

The pharmacodynamic results suggest that, the combination of high dose of Glibenclamide with *Trigonella* showed maximum hypoglycaemic effect and the effect produced by combination of low dose of Glibenclamide with fenugreek was greater than the hypoglycaemic effect produced by fenugreek alone, but was comparably less than high dose of Glibenclamide.

The popularity of herbal medicinal products (HMPs) makes it important to understand potential interactions between herbs and prescribed drugs. The likelihood of herb-drug interactions could be higher than drug-drug interactions, if only because drugs usually contain single chemical entities, while almost all HMPs (even single-herb products) contain mixtures of pharmacologically active constituents. Herbal supplements can cause potentially dangerous side effects when taken with prescription drugs and the number of cases reported for the emerging herb-drug interactions are already on the rise [24].

The pharmacokinetic results suggest that, fenugreek decreases the bioavailability of glibenclamide by inhibiting its absorption and by increasing its clearance thereby reducing the combined pharmacological effect. Hence care must be taken when the combination is prescribed for clinical benefit in diabetic patients. As fenugreek decreases the effect of glibenclamide, it may result in increase in hypoglycemia because of lower bioavailability. Hence the study of mechanisms of drug interaction is of much value in selecting drug concentrations to provide rational therapy.

The histopathological studies revealed that the combination of glibenclamide and fenugreek not only increased the volume of islets but also recovered partially destroyed beta cells. This suggests that fenugreek has good potential to be developed into hypoglycemic new dug. In the present study herb-drug interactions exist at pharmacokinetic level. Fenugreek was found to inhibit absorption of the glibenclamide and increase the renal clearance.

5. CONCLUSION

The interaction appears to be pharmacokinetic interaction at absorption and elimination. *Trigonella foenum-graecum* inhibits the absorption of which results in a significant decrease in the bioavailability later. And combination group with a lower dose of Glibenclamide produced increment to the volume of islets in pancreas compare to individual treatment. Since the interaction was seen in rats it is likely to occur in humans leading to decreased activity of glibenclamide, which may need dose adjustments. Hence care must be taken when the combination is prescribed for clinical benefit in diabetic patients. However the present study warrants further studies to find out the relevance of the interaction in human beings.

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