Influence of aqueous extract of fenugreek-seed powder on the pharmacodynamics and pharmacokinetics of gliclazide in rats/rabbits

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Background: Since drugs from oriental medicine are used along with allopathic drug therapy in highly populated countries, such as India and China, and since optimal blood-sugar control is needed in diabetes, a herbal drug that influences blood glucose was studied for its effect on gliclazide response in normal and diabetic rats and normal rabbits. **Objective:** To evaluate the safety of the herb–drug combination with respect to blood glucose in animal models. **Methods:** Blood-glucose levels were estimated by GOD/POD method using commercial glucose kits. The serum-gliclazide levels were estimated by HPLC method. Diabetes was induced in rats by intraperitoneal administration of alloxan monohydrate (100 mg and 50 mg/kg bodyweight for 2 consecutive days). **Results:** Gliclazide produced hypoglycemia in normal and diabetic rats with peak activity at 1 h and 8 h, and in rabbits at 3 h. Fenugreek-seed powder extract produced hypoglycemia when given alone and prolonged the effect of gliclazide in combination during 1–12 h in normal and diabetic rats and during 2–8 h in normal rabbits without hypoglycemic convulsions. The blood levels and pharmacokinetics of gliclazide were not altered in the presence of fenugreek extract. **Conclusion:** The study indicated that the combination can be used safely to obtain prolonged and sustained antidiabetic effect.

Keywords: fenugreek extract, gliclazide, herb–drug interaction, hypoglycemia

Use of herbs for the treatment of diabetes has been a common practice in countries such as India and China since ancient times. Such practice has now spread to developed countries, such as the USA, in the form of health supplements. In India and China, people often use both herbs and drugs together. The Chinese believe that the use of herbs along with drugs may reduce the side effects of drugs and prescribing herbs together with drugs is quite common in China. In such a situation, the herb may interact with the drug, thereby enhancing or reducing the effects of the drug, either by altering its pharmacodynamics or pharmacokinetics, or both. Literature evidence indicates that some herbs interact with oral hypoglycemic agents [1].

Gliclazide is a widely used drug in the treatment of Type 2 diabetes owing to its selective inhibitory activity towards pancreatic K^+ATP channels [2,3], antioxidant property [4], low incidence of producing severe hypoglycemia [5], and other hemobiological effects [6,7].

In the present study, the influence of aqueous extract of *Trigonella foenum-graecum* (TFG; fenugreek) seed powder on pharmacodynamics and pharmacokinetics of gliclazide in rats/rabbits was studied to evaluate the safety of the combination with respect to blood glucose. It is commonly used to treat congestion of the upper respiratory passages, to control blood sugar to treat colic, flatulence, dysentery, diarrhea, indigestion with loss of appetite, chronic cough and enlargement of the liver and spleen [8,9]. It is also reported by several authors to produce hypoglycemic and antidiabetic activities and is the active ingredient in several herbal antidiabetic formulations [10,11]. Hence, its influence on the effect of gliclazide was studied in rats and rabbits.

Experimental procedure

Pharmacodynamic study in normal/diabetic rats

Albino rats of either sex weighing between 250 and 330 g obtained from M/S. Mahaveer Enterprises, Hyderabad, India, were used in the study. They were maintained on standard pellet diet and water *ad libitum.* Animals were fasted for 18 h before the experiment and both water and food were withdrawn during the experiment. A group of six rats was administered with 2 mg/kg body weight of gliclazide, orally. The same group was administered with aqueous extract of TFG seed (30 mg/kg body weight), and the combination of extract and gliclazide, orally, with a week washout interval, was maintained between the treatments. The

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Table 1. Average percentage blood-glucose reduction with gliclazide, *Trigonella foenum graecum* and their combination in normal rats (n = 6).

Time (h)	Gliclazide	Aqueous extract of TFG	Combination
0	0	0	0
1	-43.62 ± 2.81	-5.70 ± 1.97	-49.28 ± 2.92
2	-37.74 ± 2.60	-17.27 ± 1.97	-47.54 ± 2.49*
3	-25.05 ± 2.57	-27.92 ± 2.22	-41.25 ± 2.96*
4	-28.49 ± 2.62	-22.35 ± 0.96	-43.75 ± 2.60*
6	-33.08 ± 1.83	-17.55 ± 1.11	-56.96 ± 3.00*
8	-37.89 ± 2.31	-12.83 ± 0.95	-44.02 ± 1.60*
12	-16.37 ± 1.19	-12.11 ± 1.53	-24.61 ± 2.31*

*Significant at $p < 0.05$ compared with gliclazide control.
TFG: Trigonella foenum-graecum.

same treatment was repeated in a group of six alloxan-induced diabetic rats. Blood samples were collected in rats from retro-orbital puncture at 0, 1, 2, 3, 4, 6, 8 and 12 h and were analyzed for blood glucose by GOD/POD method [12] using commercial glucose kits and semiautoanalyzer (Screen Master 3000, Mumbai, India).

Pharmacokinetic & pharmacodynamic study in normal rabbits

Normal albino rabbits of either sex weighing between 1.5 and 1.8 kg obtained from M/S. Ghosh Enterprises, Kolkata, India, were used in the study. They were maintained on a standard pellet diet and water *ad libitum*. They were fasted for 18 h before the experiment and both water and food were withdrawn during the experiment. A group of six rabbits was administered 5.6 mg/kg body weight of gliclazide, orally. The same group was administered 30 mg/kg body weight of aqueous extract of TFG seed orally, after a washout period of 1 week. After a further washout period, the same group was administered with the combination of the extract and gliclazide. Blood samples were withdrawn from the marginal ear-vein puncture of each rabbit at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h. They were analyzed for glucose by GOD/POD method [12] and for gliclazide by HPLC method, as described by Rouini and colleagues [13], and was modified in the laboratories.

The animal experiments conducted were approved by our institutional Animal Ethics Committee and by the government regulatory body for animal research (Regd No. 516 /01/A/CPCSEA).

Induction of diabetes

Diabetes was induced in rats by the administration of alloxan monohydrate in two doses of 100 mg and 50 mg/kg body weight, intraperitoneally for 2 consecutive days [14].

Table 2. Average percentage blood-glucose reduction with gliclazide, *Trigonella foenum-graecum* and their combination in diabetic rats (n = 6).

Time (h)	Gliclazide	Aqueous extract of TFG	Combination
0	0	0	0
1	-50.06 ± 1.34	-8.25 ± 0.55	-53.82 ± 1.70
2	-38.54 ± 1.16	-15.62 ± 1.18	-50.45 ± 2.22*
3	-30.99 ± 1.91	-30.08 ± 1.84	-49.52 ± 1.86*
4	-25.53 ± 0.88	-27.32 ± 0.94	-45.81 ± 2.73*
6	-34.16 ± 1.00	-18.45 ± 0.82	-51.31 ± 2.88*
8	-42.62 ± 1.45	-14.58 ± 0.90	-57.75 ± 2.40*
12	-34.01 ± 1.43	-7.56 ± 1.85	-38.55 ± 3.00

*Significant at $p < 0.05$ compared with gliclazide control.
TFG: Trigonella foenum-graecum.

Table 3. Average percentage blood-glucose reduction with gliclazide, *Trigonella foenum-graecum* and their combination in normal rabbits (n = 6).

Time (h)	Gliclazide	Aqueous extract of TFG	Combination
0	0	0	0
1	-20.58 ± 3.09	-17.84 ± 2.12	-27.21 ± 1.96
2	-27.95 ± 2.77	-29.45 ± 1.54	-45.01 ± 3.43*
3	-32.51 ± 2.73	-43.88 ± 1.39	-50.21 ± 3.16*
4	-26.73 ± 2.99	-34.13 ± 2.50	-42.45 ± 3.54*
6	-21.78 ± 2.33	-27.59 ± 2.81	-36.71 ± 3.41*
8	-15.63 ± 2.90	-19.49 ± 1.33	-28.96 ± 3.95*
12	-9.81 ± 1.27	-12.92 ± 3.16	-23.27 ± 3.94*
18	-6.92 ± 2.99	-2.29 ± 3.60	-19.86 ± 4.73*
24	-4.49 ± 2.49	0.23 ± 1.63	-15.76 ± 3.92*

**Significant at $p < 0.05$ compared with gliclazide control.*
TFG: Trigonella foenum-graecum.

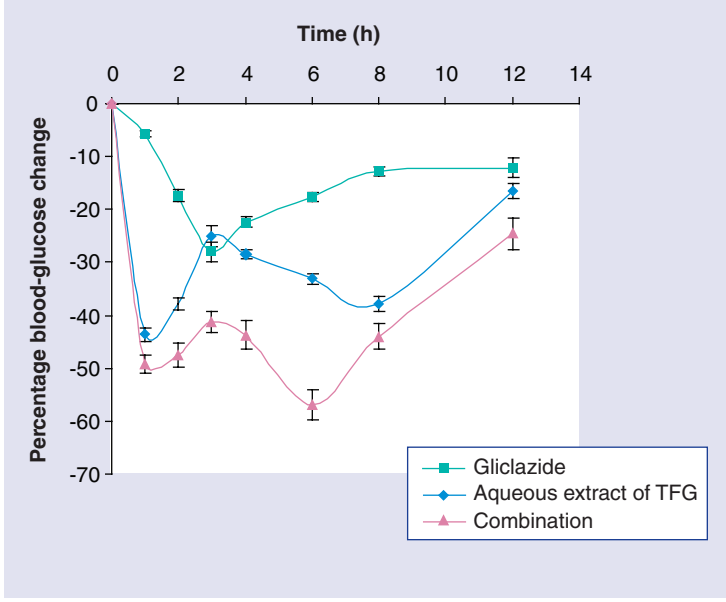
Data & statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). The significance was determined by applying Student's paired t-test. Significance was established at a p value of less than 0.05.

Results

The average percentage blood-glucose reduction with gliclazide, TFG and their combination in normal rats, diabetic rats and in normal rabbits is shown in the Tables 1, 2 & 3, respectively, and graphical representation is shown in Figures 1,2 & 3, respectively. The average serum gliclazide concentrations before and after treatment with aqueous extract of TFG in normal rabbits is shown in Table 4 and the graphical representation is given in Figure 4. The mean pharmacokinetic parameters of gliclazide before and after treatment with aqueous extract of TFG in normal rabbits is shown in Table 5.

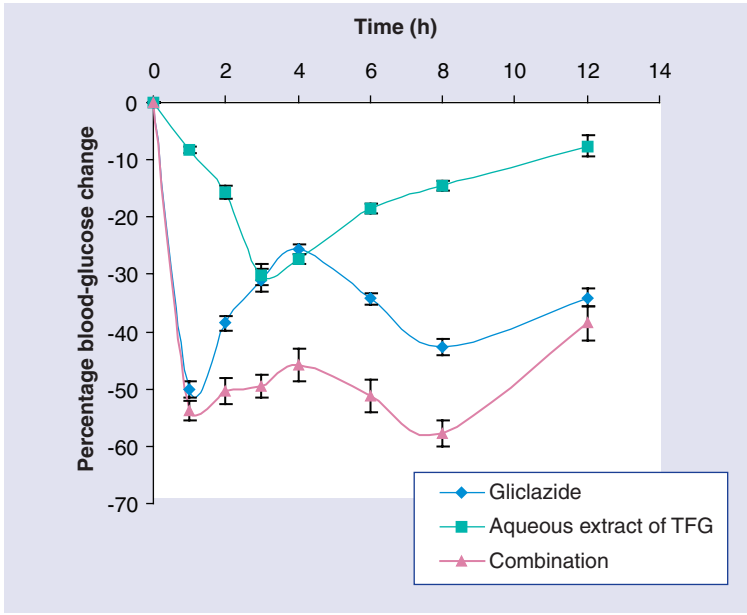
Figure 1. Percentage blood-glucose reduction with gliclazide, *Trigonella foenum-graecum* and their combination in normal rats (n = 6).



TFG: *Trigonella foenum-graecum*.

Gliclazide produced biphasic hypoglycemic activity with maximum biphasic peak reduction of 43.62 ± 2.81% and 37.89 ± 2.31% at 1 and 8 h, respectively, in normal rats, and produced antidiabetic activity with maximum percentage reduction of 50.06 ± 1.3% and 42.62 ± 1.45% at 1 and 8 h, respectively, in diabetic rats. Gliclazide produced peak activity of 32.51 ± 2.73% reduction at 3 h in normal rabbits. The aqueous extract of TFG seed alone produced 27.92 ± 2.22%, 30.08 ± 1.84% and 43.88 ± 1.39% reduction in blood glucose in normal and diabetic rats and in normal rabbits, respectively. When administered in combination, the aqueous extract of fenugreek seed significantly raised the effect of gliclazide in both rats (normal: 33.08 vs 56.96%; $p < 0.05$; diabetic: 25.53 vs 45.81%; $p < 0.05$) and rabbits (27.95 vs 45.01%; $p < 0.05$) without any convulsions. The serum gliclazide levels at 8-, 12- and 24-h intervals were decreased in the presence of aqueous extract of TFG and the pharmacokinetic parameters, such as area under the curve $(AUC)_{0–24}$, $AUC_{0-\alpha}$, area under the first moment curve $(AUMC)_{0–24}$, $AUMC_{0-\alpha}$, Kel, $T_{1/2}$, clearance and mean residence time (MRT) were altered significantly in the presence of aqueous extract of TFG in normal rabbits.

Figure 2. Percentage blood-glucose reduction with gliclazide, *Trigonella foenum-graecum* and their combination in diabetic rats (n = 6).



TFG: *Trigonella foenum-graecum*.

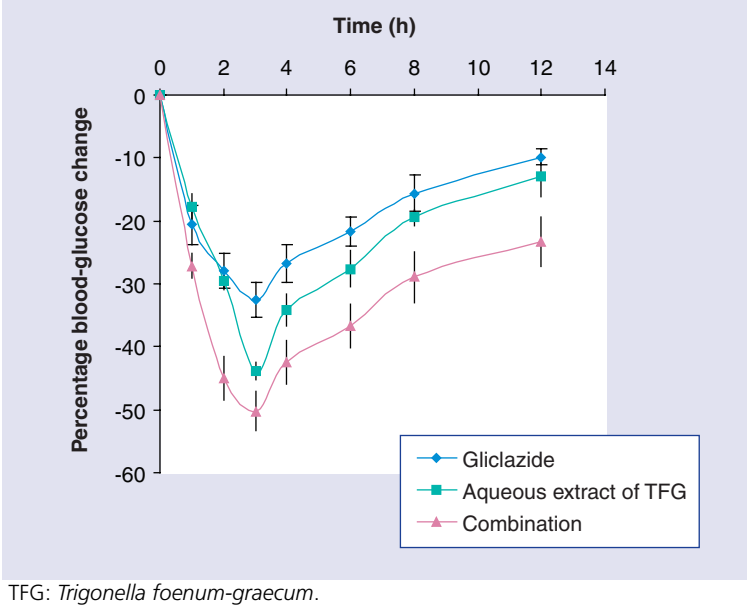
Discussion

Drug-interaction studies are usually conducted in animal models to assess the safety of the combination, before they are conducted in humans. The normal rat model served to quickly identify the interaction, and the diabetic rat model served to validate the interaction in an actual-use condition of the drugs. The rat model was used for the pharmacodynamic-interaction study, since it is the most widely used species in drug metabolism and drug interaction studies. The rabbit model is another, dissimilar, species to validate the occurrence of the interaction. The rabbit model was used for pharmacokinetic and pharmacodynamic interaction studies since a suitable quantity of blood can be obtained at different time intervals and it is the official model that is used for bioassay of insulin, other than the mouse convulsion method [15].

Gliclazide produced hyoglycemia/antihyperglycemia in normal/diabetic rats, respectively, with peak activity at 1 h and 8 h, and in rabbits at 3 h. Gliclazide is metabolized to several metabolites by hepatic cytochrome P450 3A4 and 2C9 isozymes and is eliminated in urine. A part of gliclazide is eliminated through the biliary route, which involves enterohepatic circulation in rats [16,17] and in humans [18]. The reabsorption of gliclazide eliminated through the biliary route might be responsible for a second peak in its hypoglycemic effect in rats. The absence of a second peak effect in rabbits indicates that it might not involve biliary excretion, and maybe excreted by the renal route only. Since the aqueous extract of TFG seed did not alter the pattern of double-peak effect in rats and single-peak effect in rabbit, it does not seem to interfere with the metabolic pattern of gliclazide in both species. However, the elimination kinetics of gliclazide in rabbits were found to be altered in the presence of the extract, since $T_{1/2}$ and AUC were decreased. The relatively rapid elimination of gliclazide in rabbits in the presence of the extract might be due to the change in the distribution coupled with renal elimination. The extract might not have influenced biliary excretion of gliclazide, since double-peak effects were found in the rat model in the presence, as well as absence, of the extract.

The influence of the single-dose effect of the extract on the pharmacokinetics and hypoglycemic effect of gliclazide was studied in normal rabbits to identify the main mechanisms involved in the interaction. When administered alone, gliclazide produced hyoglycemia/antihyperglycemia in normal/diabetic rats with peak activity at 1 and 8 h, and in normal rabbits at 3 h. The aqueous extract of fenugreek seed produced significant hypoglycemia when administered alone, and prolonged the effect of

Figure 3. Percentage blood-glucose reduction with gliclazide, *Trigonella foenum-graecum* and their combination in normal rabbits (n = 6).



TFG: *Trigonella foenum-graecum*.

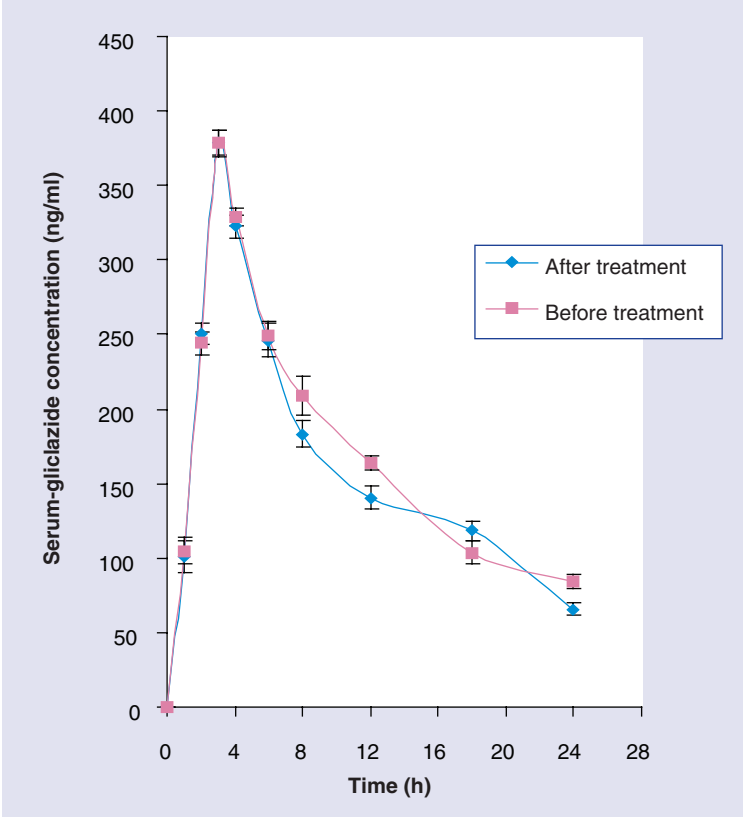
Table 4. Average serum gliclazide concentrations before and after treatment with aqueous extract of *Trigonella foenum-graecum* in normal rabbits (n = 6).

Time (h)	Serum gliclazide concentration (mean ± SEM) (ng/ml)		Significance at $p < 0.05$
	Before treatment	*After treatment*	
0	0	0	
1	104.88 ± 8.58	100.88 ± 10.88	NS
2	244.14 ± 7.98	250.17 ± 7.30	NS
3	378.51 ± 8.71	378.60 ± 8.29	NS
4	328.82 ± 6.31	322.57 ± 7.49	NS
6	249.27 ± 9.69	246.02 ± 11.34	NS
8	209.13 ± 13.07	183.20 ± 8.83	S
12	163.98 ± 5.03	140.55 ± 8.12	S
18	103.64 ± 7.40	118.33 ± 6.17	NS
24	84.63 ± 4.71	65.77 ± 3.85	S

NS: Not significant; S: Significant; SEM: Standard error of the mean.

gliclazide by 1–12 h in normal and diabetic rats, and by 2–8 h in normal rabbits, without hypoglycemic convulsions. TFG extract produced hypoglycemia/antihyperglycemia and its influence in gliclazide activity in rats/rabbits may be caused by its insulin-secreting action. In rabbits, relatively rapid elimination of gliclazide is expected to reduce its response. However, since gliclazide and the extract possessed hypoglycemic activity, the combined effect appears to overcome the effect of relatively rapid elimination of gliclazide. The observed interaction appears to be mainly pharmacodynamic, and partly pharmacokinetic, in nature. It is well established that gliclazide (sulphonylurea derivative) produces hypoglycemia by pancreatic (insulin release) and extrapancreatic (increase in glucose uptake) mechanisms. The hypoglycemic/antihyperglycemic effect of TFG might be due to its effect on insulin release. Literature reports by several authors indicate that a peculiar amino acid, 4-hydroxy isoleucine, isolated from TFG seed, was found to have insulinotrophic activity, which might be responsible for decreased blood-glucose levels with aqueous extract of TFG. These findings support our results [19,20]. Hence, the enhanced effect of gliclazide in the presence of aqueous extract of TFG might be due to the combined effect of the two drugs on insulin release. However, no convulsions were seen in rats or rabbits, even at peak hours of activity. Hence, their combination need not be contraindicated.

Figure 4. Average serum gliclazide concentrations before and after treatment with aqueous extract of *Trigonella foenum-graecum* in normal rabbits (n = 6).



Conclusion

The study indicates that the combination of gliclazide and aqueous extract of TFG can be used safely with respect to blood glucose to obtain a prolonged and sustained antidiabetic effect. Further studies are needed to establish its long-term safety in animals and humans.

Table 5. Mean pharmacokinetic parameters of gliclazide before and after treatment with aqueous extract of *Trigonella foenum-graecum* in normal rabbits (n = 6).

Kinetic parameter	Pharmacokinetic parameters of gliclazide (mean ± SEM)		Significance at $p < 0.05$
	Before treatment	***After treatment***	
$AUC_{0–24}$ (ng/ml/h)	4096.82 ± 117.74	3541.91 ± 217.76	S
$AUMC_{0–24}$ (ng/ml/h*h)	56153.16 ± 2497.35	36054.86 ± 3073.26	S
Kel (h^{-1})	0.05 ± 0.00	0.08 ± 0.00	S
$AUC_{0-\alpha}$ (ng/ml/h)	5755.90 ± 234.33	4199.11 ± 271.76	S
$AUMC_{0-\alpha}$ (ng/ml/h*h)	128125.10 ± 10868.39	60316.16 ± 5943.50	S
$T_{1/2}$ (h)	13.06 ± 0.84	8.81 ± 0.45	S
K_a (h^{-1})	1.54 ± 0.00	1.54 ± 0.00	NS
Clearance (ml/h)	911.78 ± 31.71	1368.11 ± 49.30	S
Clearance (ml/h/kg)	653.51 ± 26.45	906.54 ± 55.42	S
Vdss (ml)	19420.22 ± 716.25	18645.39 ± 1214.38	NS
Vdss (ml/kg)	13901.66 ± 498.16	12310.58 ± 883.35	NS
Vdarea (ml)	17055.83 ± 784.78	17411.41 ± 1200.06	NS
Vdarea (ml/kg)	12211.07 ± 566.79	11524.37 ± 920.60	NS
MRT (h)	22.10 ± 1.14	14.29 ± 0.76	S
C_{max} (ng/ml)	380.52 ± 7.42	357.69 ± 12.75	NS
T_{max} (h)	3.00 ± 0.00	3.00 ± 0.00	NS

AUC: Area under the curve; AUMC: Area under the first movement curve; MRT: Mean residence time; NS: Not significant; S: Significant; SEM: Standard error of the mean; Vdarea: Area of volume of distribution; Vdss: Steady state volume of distribution.

Future perspective

Currently, polypharmacy, that is, the use of drugs along with herbal preparations, is quite common practice throughout the world for better and more effective therapy. In such a situation, the drug–herb interaction studies are helpful to establish the safety of the drug therapy. This study provides safety data regarding the use of TFG along with gliclazide in patients with chronic diabetes in order to get better therapeutic effect. Similar studies will help to prevent the unwanted effects produced by drug–herb interactions.

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Executive summary

- The influence of aqueous extract of *Trigonella foenum-graecum*-seed powder on the pharmacodynamics and pharmacokinetics of gliclazide in rats/rabbits was studied.
- Gliclazide produced hypoglycemia in normal and diabetic rats, with peak activity at 1 h and 8 h, and in rabbits at 3 h.
- The aqueous extract of TFG-seed powder produced hypoglycemia when given alone and prolonged the effect of gliclazide in combination by 1–12 h in normal and diabetic rats, and by 2–8 h in normal rabbits, without hypoglycemic convulsions.
- The serum gliclazide levels at 8-, 12- and 24-h intervals were decreased in the presence of aqueous extract of TFG and pharmacokinetic parameters, such as area under the curve $(AUC)_{0–24}$, $AUC_{0-\alpha}$, area under the first moment curve $(AUMC)_{0–24}$, $AUMC_{0-\alpha}$, Kel, $T_{1/2}$, clearance and mean residence time, were altered significantly in the presence of aqueous extract of TFG in normal rabbits.
- The interaction observed appears to be mainly pharmacodynamic and partly pharmacokinetic in nature.
- The study indicated that the combination can be used safely to get prolonged and sustained antidiabetic effect.

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