

## **HYPOGLYCEMIC EFFECT OF TRIGONELLINE ISOLATED FROM IRAQI FENUGREEK SEEDS IN NORMAL AND ALLOXAN-DIABETIC RABBITS**

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### **Abstract**

Fenugreek (*trigonella foenum graecum*) is one of the most widely used medicinal plants in folk medicine. It is known to have a diuretic, cardio tonic, hypotensive, hypoglycemic and hypolipidemic effect. This study was designed to evaluate the hypoglycemic effect of Trigonelline isolated from Iraqi fenugreek seeds in normal and alloxan –diabetic rabbits.

Sixty male adult albino rabbits were used in this study. They were divided randomly into two main groups, a non-diabetic group (group A) and a diabetic group (group B). The two groups were further subdivided into subgroups, A1 - A4 and B1 – B5. Group A1 & B1 were control. Group A2 & B2, A3 , B3 and A4 & B4 were received orally 10mg/12hrs isolated pure trigonelline, 10mg/12 hrs standard Trigonelline and 7.7ml/12hrs ethanol extract of the fenugreek seeds respectively, while group B5 received insulin.

The data revealed that the isolated pure trigonelline exhibited a significant ( $P < 0.05$ ) hypoglycemic effect in normal and diabetic rabbits, but its effect is more profound in diabetic groups. Isolated pure trigonelline reduces blood glucose level by 21.3% in the non-diabetic

group and 27.53% in the diabetic group. The result of this study also indicated that the hypoglycemic effect of ethanol extract of fenugreek seeds and that of isolated pure trigonelline was not significant ( $P > 0.05$ ).

In conclusion, based on the result of this study, trigonelline at a dose of 10mg/12hrs has a significant hypoglycemic effect in both normal and diabetic rabbits. It is preferred to use trigonelline to avoid the possible adverse reactions which may appear due to the multi-components of the ethanol extract of the fenugreek seeds.

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**Keywords:** Trigonella foenum graecum seeds, isolated trigonelline alkaloids, alloxan rabbit

### **Introduction**

Seeds of fenugreek (*trigonella foenum graecum*) have a wide range of non-therapeutic uses. It is used as a natural source of food flavoring (1). Fenugreek is one of the most medicinal plants which is widely used in folk medicine. It has a diuretic, uterine & cardio tonic, hypotensive, hypolipidemic, hypoglycemic, hyperinsulinemic, antidiabetic effects, antinociceptive and anti-inflammatory (1-4). Type II diabetes is a disease characterized by chronic hyperglycaemia and oxidative stress (5). *Trigonella foenum-graecum* Linn. (Leguminosae) has been extensively used as a source of antidiabetic compound, from its seeds, leaves and extracts in different model systems (6). The chemical constituents of TSP include volatile oils, alkaloids, saponins, saponogenins, flavonoids and mucilage (6). The antidiabetic effect of fenugreek has been the subject of different articles; many studies have been shown that many substances in the seeds possess hypoglycemic effects (1,5-7, 10, 11). This study was designed to evaluate the hypoglycemic effect of trigonelline, the major alkaloid, isolated from the seeds of Iraqi fenugreek in normal and alloxan-diabetic rabbits

### **Experiment**

#### **A- Materials:**

Trigonelline was isolated from Iraqi fenugreek seeds according to the method of Suhad et al. The ethanol extract of fenugreek seeds was prepared by refluxing the defatted fenugreek seeds for two hours (12). Standard trigonelline was purchased from Fluka Chemical. Glucose Kit was obtained from Lab Kit (Spain)

#### **B- Animal study:**

Fifty three male adult albino rabbits were used in this study and divided randomly into two main groups, a non-diabetic group (group A) (N=24). The other 29 rabbits were made diabetic by a single intraperitoneal injection of 75mg/kg of alloxan monohydrate at weekly intervals following 12 h fasting (13, 19) and assigned as diabetic group (group B); diabetes was confirmed by the presence of glucosuria and hyperglycemia. The two groups were further

subdivided into subgroups, A1 – A4 and B1 – B5. Groups A1 & B1 were control and received distilled water. Groups A2 & B2, A3 & B3, and A4 & B4 were orally received 10mg/12 hours isolated trigonelline, 10mg/12 hours standard trigonelline and 7.7ml/12 hours ethanol extract of the fenugreek seeds respectively as shown in figure 1. Group B5 received insulin as insulin zinc suspension [Monotard®] which was administered at a dose of 10 IU/day subcutaneously (14). Treatment duration in all groups was for four weeks.

Fasting blood samples were collected from the middle ear artery at zero time (just before starting the treatment) and after 7, 14, and 28 days. Xylol was used to facilitate blood sampling. Blood samples were placed into plastic tubes without heparin for centrifugation and the serum was separated. Serum samples were stored at -20C until the analysis were performed.

#### C- Dosing & dosage regimen:

In most clinical studies, the mean dose of fenugreek seeds used was 15gm of seeds or seeds powder per day (15), therefore the dose of trigonelline and the ethanol extract of the seeds has been selected to be equivalent to 15gm of seeds powder. It was 20mg of trigonelline and 15.4ml of the ethanol extract per day. Further more, trigonelline was given in four different doses (1, 3, 5, and 10mg/12hrs) to 20 normal rabbits. It was noticed that 10mg gave the best hypoglycemic effect which lasted for more than 9 hours as shown in figure 1. Therefore, trigonelline was given at a dose of 10mg/12hrs and the ethanol extract was given at a dose of 7.7ml/12hrs.

Serum glucose was determined using lab kit for glucose, according to the method of Barham & Trindoe (16). Glucose was determined after enzymatic oxidation of glucose by glucose oxidase to form hydrogen peroxide and gluconate. Hydrogen peroxide then reacts with phenol and 4-aminophenazone in the presence of peroxidase to form quinoimine. The absorbance at 505nm was determined against blank reagent and the results were expressed as mg/dl, based on standard glucose solution treated in the same manner.

#### **Results and discussion:**

Results of 10mg of trigonelline exhibited a significant hypoglycemic effect ( $P < 0.05$ ) which lasted for 9 hours as shown in figure 1. The results differ from those obtained by Shani et al (17) who stated that trigonelline has a mild and transient hypoglycemic effect.

In normal rabbits (group A), after four weeks of treatment, the isolated trigonelline (I.T.), standard trigonelline (S.T.), and ethanol extract (E.E.) of fenugreek seed significantly reduced serum glucose ( $P < 0.05$ ) when compared with the pretreatment values as illustrated in table 1. The percent reduction in serum glucose level was 22.34%, 21.03% and 27.55% (table

2) for isolated trigonelline, standard trigonelline and ethanol extract respectively as shown in figure 2. There is a non-significant difference among the effect of the three types of treatment.

In diabetic rabbits (group B), after one month of the alloxan treatment, fasting blood glucose level was significantly elevated ( $P < 0.05$ ) from the pretreatment values which agreed with those described by Bell et al (18). All types of treatment (isolated trigonelline, standard trigonelline, and ethanol extract) significantly ( $P < 0.05$ ) reduced blood glucose level from the first week as shown in table 3. After four weeks, the percent reduction in blood glucose was 27.05%, 27.53% and 32.61% (table 4) for isolated trigonelline, standard trigonelline, and ethanol extract respectively (figure 3). Although there is a non-significant difference between hypoglycemic effect of isolated trigonelline & standard trigonelline ( $P > 0.05$ ), there is a significant difference ( $P < 0.005$ ) between the effect of ethanol extract and both isolated trigonelline & standard trigonelline.

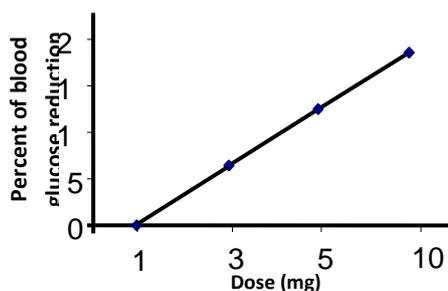
The results of this study revealed that isolated trigonelline from Iraqi fenugreek seeds exhibited a significant hypoglycemic effect in normal and diabetic rabbits but its effect was more profound in the diabetic group. It reduces blood glucose by 22.34% in the non-diabetic and 27.53% in the diabetic group.

The hypoglycemic effects of ethanol extract of the seeds was more potent than that of Isolated trigonelline which may be due to synergistic effect of many hypoglycemic substances present in the extract like nicotinic acid, Fenugreekine, coumarine, minerals and fibers. Despite the beneficial effect of these substances in the extract, they may carry the risk of possible adverse reaction. Therefore, isolated trigonelline may be preferred to be used as hypoglycemic agent.

## **Conclusion**

In the present study it was observed that trigonella possess significant blood sugar lowering activity in alloxan-induced diabetic rabbit. The hypoglycemic activity of isolated trigonelline was compared with standard trigonelline, ethanol extract and insulin.

In conclusion, isolated trigonelline at a dose of 10mg/12hrs has a promising hypoglycemic effect in diabetic rabbits.



**Figure (1):** Effect of 1mg, 3mg, 5mg and 10mg of the isolated trigonelline on blood glucose in normal rabbits after 1hour of each dose.

**Table (1):** Effect of I.T. [10mg/12hr p.o.], S.T. [10mg/12hr p.o.] and E.E. of fenugreek seeds [7.7ml/12hr p.o.] on blood glucose conc. [mg/dl] in normal rabbits during four weeks.

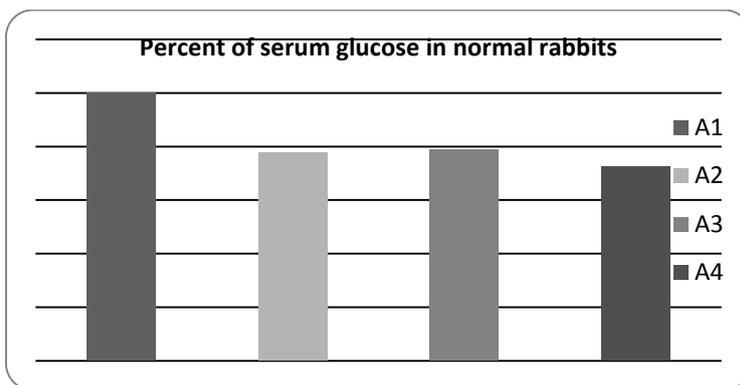
Animal Group	Serum glucose conc.[mg/dl] mean±SEM				
	Zero time	1st week	2nd week	3rd week	4th week
A1	95 ± 7	104 ± 7	97 ± 5	97 ± 7	93 ± 8
A2	94 ± 6	77 ± 6	75* ± 7	75* ± 9	73* ± 8
A3	108 ± 8	93* ± 8	91* ± 9	89* ± 9	85* ± 6
A4	98 ± 3	77* ± 8	77* ± 10	75* ± 8	71* ± 5

SEM: standard error of the mean.

\* : significant difference with respect to pretreatment. [p<0.05].

**Table (2):** Percent of reduction in blood glucose in normal rabbits during four weeks treatment with I.T. [10mg/12hr p.o.], S.T. [10mg/12hr p.o.] and E.E. of fenugreek seeds [7.7ml/12hr p.o.].

Animal Group	Percent of reduction in serum glucose				
	Zero time	1st week	2nd week	3rd week	4th week
A1	0	0	0	0	0
A2	0	18.09	20.21	20.21	22.34
A3	0	13.89	15.74	17.59	21.30
A4	0	21.43	21.43	23.47	27.55



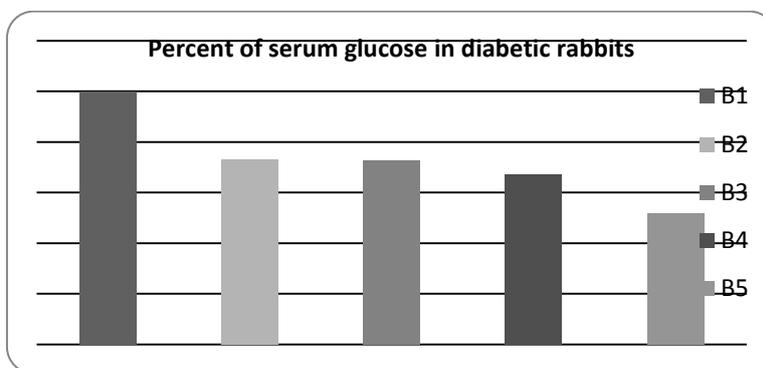
**Figure (2): Percent of serum glucose in normal rabbits after four weeks treatment**

Where: A1: control.

A2: received I.T.

A3: received S.T.

A4: received E.E.



**Figure (3): Percent of serum glucose in diabetic rabbits after four weeks treatment**

Where: B1: control.

B2: received I.T.

B3: received S.T.

B4: received E.E.

B5: received insulin.

**Table (3): Effect of I.T. [10mg/12hr p.o.], S.T. [10mg/12hr p.o.], E.E. of fenugreek seeds [7.7ml/12hr p.o.] and insulin [10 IU/day s.c.] on blood glucose conc. [mg/dl] in diabetic rabbits during four weeks.**

Animal Group	Serum glucose conc.[mg/dl] mean ± SEM				
	Zero time	1st week	2nd week	3rd week	4th week
B1	368 <sup>•</sup> ± 21	386 ± 17	385 ± 17	403 ± 22	413 ± 21
B2	436 <sup>•</sup> ± 22	339* ± 8	328* ± 20	320* ± 20	318* ± 21
B3	385 <sup>•</sup> ± 24	302* ± 8	292* ± 22	281* ± 19	279* ± 15
B4	463 <sup>•</sup> ± 16	321* ± 18	316* ± 24	309* ± 15	312 <sup>▶</sup> * ± 14
B5	365 <sup>•</sup> ± 29	197* ± 19	214* ± 21	186* ± 22	189 <sup>‡</sup> * ± 24

SEM:- standard error of the mean.

- \* :- significant difference with respect to pretreatment [p<0.05].
- ‡ :- significant difference between B5 and B1, B2, B3 and B4 [p < 0.05]..
- :- significant difference with respect to non-diabetic rabbits [P<0.05].
- ▶ :- significant difference between B4 & B1, B2 and B3 [P<0.05].

**Table (4): Percent of reduction in blood glucose conc. in diabetic rabbits during four weeks treatment with the I.T. [10mg/12hr p.o.], S.T. [10mg/12hr p.o.], E.E. of fenugreek seeds [7.7ml/12hr p.o.] and insulin [10 IU/day s.c.].**

Animal Group	Percent of reduction in serum glucose				
	Zero time	1st week	2nd week	3rd week	4th week
B1	0	0	0	0	0
B2	0	22.25	24.77	26.61	27.06
B3	0	21.56	24.16	27.01	27.53
B4	0	30.67	31.75	33.26	32.61
B5	0	46.03	41.37	49.04	48.22

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