

BIOCHEMICAL EFFECTS OF *TRIGONELLA FOENUM- GRAECUM* L. SEEDS IN NORMAL AND ALLOXAN INDUCED DIABETIC RABBITS

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ABSTRACT

This study was undertaken to investigate the effect of ethanolic extract of *Trigonella foenum-graecum* L. (fenugreek) seeds on blood glucose, total serum cholesterol and total serum protein in normal and alloxan induced diabetic rabbits.

Twenty-four mature male rabbits (local breed) were randomly divided into four groups of equal number: (Control negative group (normal rabbits received 2 ml normal saline). Normal animals group treated orally with (200 mg /kg b.w.) alcoholic extract of fenugreek daily. Diabetic positive control group (induced diabetic rabbits received 2 ml normal saline). Diabetic group treated orally with (200 mg /kg b.w.) alcoholic extract of fenugreek daily. All groups were deranged for six weeks. Diabetes was induced experimentally by single intravenous injection of alloxan monohydrate (100 mg/ kg b.w.). Estimation of blood glucose, total cholesterol and total protein was carried out weekly for all groups of the experiment.

The results showed that alcoholic extract of *T. foenum-graecum* L. caused significant decrease ($P < 0.05$) in the levels of serum glucose and total cholesterol in diabetic treated groups after one week of administration. Non significant decrease in blood glucose concentration of normal treated rabbits compared with normal control group. Moreover significant increases ($P < 0.05$) in total serum protein in diabetic treated groups.

This study concluded that the alcoholic extract of *T. foenum-graecum* can lower blood glucose, total serum cholesterol and improve total serum protein.

INTRODUCTION

There are many anti-diabetic plants, which might provide useful source for the development of drugs in the treatment of diabetes mellitus, such as *Trigonella foenum-graecum* (fenugreek) has along history of medical uses (1,2). In India the seeds of fenugreek are commonly consumed by people suffering from diabetes. An anti-diabetic effect of fenugreek has been demonstrated experimentally induced in dogs, rats and mice (3,4,5).

In animal and several human trials fenugreek seeds have been found to lower fasting serum glucose level both acutely and chronically (6). It has been reported also that fenugreek acts by delaying glucose absorption and enhancing its utilization (7). Moreover, the effect of fenugreek on glucose uptake and utilization has been studied in peripheral tissues (8).

Neeraja and Rajyalakshmi (9) presented a poorly designed, complex case series including six men with type 2 diabetes and six without diabetes the cases suggest that fenugreek reduced postprandial hyperglycemia primarily in subjects with diabetes but less so in subjects without diabetes. They also reported that this might be more pronounced if raw seeds rather than boiled seeds had been used.

Gupta *et al.* (10) suggested in their study that fenugreek seeds extract and diet / exercise may be equally effective strategies for attaining glycemic control in type 2 diabetes.

Several case series have also found hypocholesterolemic effects associated with oral fenugreek. Sharma *et al.* (4) noted that a small but statistically significant reduction in total cholesterol and low-density lipoprotein cholesterol levels (LDL-C), but the level of high-density lipoprotein cholesterol (HDL-C) remained unchanged. In another study, Sharma (11) also reported a decrease in the total cholesterol levels in five diabetic patients treated with fenugreek seed powder (25 gm) orally per day for 21 days. Bordia *et al.* (12) studied the effect of fenugreek seed powder on the subject who had coronary artery disease and type 2 diabetes, they noted change in the (HDL-C) level.

The present study was conducted to determine the effect of oral administration of alcoholic extract of *Trigonella foenum-graecum* (fenugreek) seeds in diabetic and normal rabbits on blood glucose level, total serum proteins and total serum cholesterol.

MATERIALS AND METHODS

Plant preparation: Seeds of *Trigonella foenum-graecum* (fenugreek) were purchased from local markets in Basrah Province / Iraq. Voucher specimens were deposited to be identified and authenticated at College of Science / University of Basrah.

Preparation of alcoholic extract: The seeds of *T. foenum-graecum* were cleaned, washed and dried at room temperature. Seeds were grounded for 2 minutes by electrical grinder.

Twenty five grams of ground seeds powder were refluxed with 250 ml (ethanol 70%) for 12 hours by Soxhlete, and then filtered by using Buchner funnel and filter paper. The solvent was dried and concentrated by using rotary evaporator at 50°C. The final dryness was done by leaving residue in room temperature (13). The resultant extract (2.4gm) was deep yellow and viscous liquid. The extract was kept in dark glass container at 4 °C.

Experimental animals: Twenty-four healthy male domestic rabbits (*Lepus cuniculus*) were brought from the local markets / Basrah, weighing (1300-1800) grams. Before using the rabbit for the experiment, the rabbits were kept under observation for a week in animal house of the College of Veterinary Medicine / University of Basrah. The animals were offered a balanced rabbit's diet that consists of green leaves, fodder and water *ad libitum* and given a prophylaxis drug against coccidiosis (Amprolium 1 g/L of drinking water).

Induction of diabetes mellitus: Diabetes mellitus was induced in overnight fasting rabbits by a single injection of alloxan monohydrate (100 mg / kg b.w) marginal ear vein. Each 100 mg of alloxan was diluted in 1 ml of normal saline (Al-Aumar, 1994). Immediately after alloxan injection, 10 ml of (20% glucose) was injected to the rabbits in order to overcome sudden decrease in blood glucose level (hypoglycemia). The rabbits were prevented from feeding for 12 hours and water replaced by 5% glucose for 24 hours. The procedures of the administrations and blood collection made under sedation of animals by using Ketamin 44 mg / kg body weight and Xylazine 5 mg / kg (15).

Experimental design: Twenty four male rabbits were divided into four groups (6 rabbits for each group) as follow. **Group I:** normal rabbits are served as control which are treated with 2 ml of normal saline (0.85 % NaCl) daily (for six negative control group). **Group II:** normal rabbits were treated with 200 mg / kg b.w fenugreek alcoholic extract orally daily for 6 weeks. **Group III:** diabetic rabbits were treated with 2 ml of normal saline orally daily (positive control group). **Group IV:** diabetic rabbits were treated with 200 mg / kg body weight fenugreek alcoholic extract orally daily for 6 weeks(16).

Blood sampling for Biochemical measurements: In every week the blood samples were collected from ear margin vein by using syringe (2 ml) and poured into test tubes. Then the blood samples were centrifuged at (5000 rpm) for 10 minutes to isolate blood serum to estimate the biochemical measurement such as glucose, total cholesterol and total protein.

Biochemical measurements:

Glucose: The serum glucose was measured by using glucose-oxidase method (17), (Biomaghreb / GOD – PAP, Tunisia).

2. Total cholesterol: The serum total cholesterol (TC) was enzymatically measured by using a commercial kit (Spinreact / CHOD – POD, Spain) as reported by (18).

3. Total protein determination: The serum total protein was estimated by the photometric colorimetric test for total proteins / Biuret method by using special kit (Human / Total protein liquicolor, Germany).

The statistical analysis: The results of the present study were analyzed by using two-way covariance (ANOVA) test in all study. All statistical calculations were carried out by the aid of the statistical package SPSS V. 11 (SPSS Inc.). The data were expressed as means \pm standard error ($X \pm SE$). Least significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) SPSS (1998).

RESULTS

The effect of alloxan injection (100 mg/kg) body weight on the blood glucose concentration in the diabetic positive control and diabetic treated groups display in Table (1). These groups showed significant increase ($P < 0.05$) in serum glucose in

week zero when compared with the pretreatment period and the negative control group.

The effect of daily oral administration (200 mg / kg b.w) of alcoholic extract of *T. foenum-graecum* on the blood glucose concentration in rabbits shows significant decrease ($P<0.05$) in diabetic treated group as compared with diabetic positive control group after one week of the administration and during the period of experiment, this reduction was proportional with the time. On the other hand, there were no statistical differences between normal treated rabbits received (200 mg /kgb.w) of alcoholic extract of *T. foenum-graecum* and negative control received (0.85% NaCl).

Effect of alcoholic extract on serum total cholesterol: Table (2) shows the effect of alcoholic extract of *T. foenum graecum* seeds on the serum total cholesterol. During the treatment period a significant decrease ($P<0.05$) in the serum total cholesterol concentration was detected in the diabetic animals dosed with (200 mg / kg body weight) of alcoholic extract when compared with the diabetic control group. There were no significant differences (N.S.) in the total cholesterol between the normal control group and the normal animals treated with alcoholic extract group.

Effect of alcoholic extract on serum total protein: The results of the effect of *T. foenum-graecum* seeds on serum total protein showed a significant increase ($P<0.05$) in the diabetic treated group as compared with the diabetic control group in the 4th, 5th and 6th week of administration (Table 3).

Table (1): Effect of alcoholic extract of *Trigonella foenum graecum* on blood glucose level in diabetic and normal rabbits.

FASTING BLOOD GLUCOSE CONCENTRATION mg/dl				
GROUPS WEEKS	NORMA L (-ve) CONTR OL	NORMA L TREAT ED	DIABET IC (+ve) CONTR OL	DIABET IC TREAT ED
PRE- TREATME NT *	103.5± 1.99 Aa	110.18±2 .66 Aa	107.41±3 .69 Af	109.71±3 .42 Ac
0WK \$	107.5±3. 04 Ca	99.8±2.2 4 Ca	231±11.1 5 Be	268±7.5 Aa
1WK #	104.78±3 .51 Ca	103.26±3 .57 Ca	251.83±1 4.3 Ad	209.23±9 .06 Bb
2WK	97.56±0. 85 Ca	99.5±1.4 7 Ca	303.5±10 .14 Ac	152.93±7 .6 Bc
3WK	103±3.2 Ca	96±1.71 Ca	355.3±16 .25 Ab	137.16±5 .12 Bc
4WK	101.25±3 .74 Ca	96.83±2. 05 Ca	397.16±9 .25 Aa	131.5±1. 87 Bc
5WK	96.51±1. 06 Ca	95.66±1. 7 Ca	366.8±6. 6 Ab	123.3±2. 37 Bc
6WK	105.4±3. 56 Ba	95.68±2. 72 Ba	379.3±4. 38 Ab	110.3±2. 61 Bc

Values are expressed as mean ± SE., n = 6 / group.

Capital letters denote differences between groups, P< 0.05 vs. control.

Small letters denote differences within groups, P< 0.05 vs. control.

(PT= pre-treatment, 0WK= one week after alloxan injection, 1WK= one week after the beginning of treatment with fenugreek).

Table (2): Effect of alcoholic extract of *Trigonella foenum graecum* on serum total cholesterol concentration

TOTAL CHOLESTEROL CONCENTRATION mg/dl				
GROUPS WEEKS	NORMAL (-ve) CONTROL	NORMAL TREATED	DIABETIC (+ve) CONTROL	DIABETIC TREATED
PRE- TREATMENT *	106.16±6.67 Aa	109.83±8.31 Aa	116.5±8.33 Ab	114.66±7.71 Aa
0WK \$	106.83±6.08 Ba	112.83±8.49 Ba	147.83±14.24 Ab	151.5±2.44 Aa
1WK #	105.66±6.5 Ba	106.33±3.37 Ba	159.66±9.51 Ab	159.16±6.68 Aa
2WK	104.16±7.18 Ba	104.83±4.9 Ba	156.33±11.42 Ab	154.66±7.67 Aa
3WK	110.16±6.14 Ca	102.16±4.97 Ca	215.33±25.04 Aa	147.33± 8.35 Ba
4WK	107.33±6.08 Ca	102.83±5.9 Ca	236±28.5 Aa	143.66±4.8 Ba
5WK	108.5±9.07 Ba	101.83±1.85 Ba	240.33±29.25 Aa	131±5.41 Ba
6WK	105±5.44 Ba	133±3.08 Ba	249.16±25.9 Aa	119.66±562 Ba

Values are expressed as mean ± SE., n = 6 / group.

Capital letters denote differences between groups, P< 0.05 vs. control.

Small letters denote differences within groups, P< 0.05 vs. control.

(PT= pre-treatment, 0WK= one week after alloxan injection, 1WK= one week after the beginning of treatment with fenugreek).

Table (3): Effect of alcoholic extract of *Trigonella foenum- graecum* on serum total Protein concentration

SERUM TOTAL PROTEIN CONCENTRATION gm/dl				
GROUPS WEEKS	NORMAL (-ve) CONTROL	NORMAL TREATED	DIABETIC (+ve) CONTROL	DIABETIC TREATED
PRE- TREATMENT *	7.1± 0.17 Aa	6.96± 0.09 Aa	6.95± 0.07 Aa	7.01± 0.11 Aa
0WK \$	6.93± 0.98 Aa	7±0.17 Aa	5.43± 0.14 Bb	5.3± 0.15 Bb
1WK #	6.96± 0.21 Aa	6.96± 0.08 Aa	5.2± 0.09 Bb	5.5± 0.15 Bb
2WK	7.03± 0.84 Aa	6.95± 0.13 Aa	5.23± 0.07 Cb	5.96± 0.12 Bb
3WK	6.86± 0.98 ABa	7.01± 0.15 Aa	5.28± 0.11 Cb	6.51± 0.19 BCb
4WK	6.78± 0.114 Aa	6.98± 0.18 Aa	5.18± 0.07 Bb	7± 0.14 Aa
5WK	6.83± 0.17 Aa	7.03± 0.7 Aa	5.18± 0.11 Bb	7± 0.14 Aa
6WK	7.05± 0.2 Aa	7± 0.05 Aa	5.21± 0.1 Bb	7.21± 0.17 Aa

Values are expressed as mean ± SE., n = 6 / group.

Capital letters denote differences between groups, P< 0.05 vs. control.

Small letters denote differences within groups, P< 0.05 vs. control.

(PT= pre-treatment, 0WK= one week after alloxan injection, 1WK= one week after the beginning of treatment with fenugreek)

DISCUSSION

Effect of treatment with alloxan on blood glucose concentration

Alloxan is mostly used to induce diabetes mellitus in rabbits (19). The dose used in this experiment 100 mg/kg body weight was clinically effective and led to induced hyperglycemia within three days (20). The results revealed significant increase in serum glucose concentration in the diabetic groups compared with the control group, which is consistent with (14,21,22,23,24) who gave nearly similar results by using this dose.

Effect of alcoholic extract on blood glucose concentration

The results of the present study revealed significant decrease in blood glucose concentration in diabetic group treated for 6 weeks with fenugreek (200 mg/kg body weight). Other scientific reports have demonstrated that fenugreek seeds can lower blood glucose in type 1 and type 2 diabetes in humans and the experimental diabetic animals (dogs, mice, rats and rabbits) (1,2,3,25,26,27,28,29,30,31,32,33).

Madar and Throne (34) attributed the hypoglycemic effect of fenugreek to dietary fibers present in the fenugreek seeds, which help in the management of metabolic abnormalities associated with diabetes such as peripheral insulin resistance and lipid abnormalities. Petit *et al.* (35) and Yoshikawa *et al.* (36) claimed that, trigonoid Ia, Ib, IIa, IIb, IIIa, IIIb, glucosides and trifoenoside A are the active principals owing to there hypoglycemic effects.

On the other hand, Sauvaire *et al.* (37) and Broca *et al.* (38) have demonstrated evidence of insulinotropic and antidiabetic properties of 4- hydroxyisoleucine isolated from fenugreek seeds. They suggested that antidiabetic effect of 4- hydroxyisoleucine was, at least in part, from direct pancreatic beta cell stimulation.

Other studies showed that the fenugreek seeds delayed gastric emptying and caused the inhibition of glucose transport as the seed contains around 50% pectin that forms a colloid suspension when hydrated can decrease the rate of gastric emptying and slow carbohydrate absorption (39,40).

While Hannan *et al.* (41) concluded that fenugreek seeds exert hypoglycemic effects by stimulating glucose dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activity of α -amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism. Moreover, Vijayakumar *et al.* (42) suggested that the hypoglycemic effect of fenugreek seeds extract partly mediated, by the activation of an insulin-signaling pathway in adipocytes and liver cells. However, one can be sure which factor that discussed before causes the reduction in glucose level.

The present study indicates that the administration of (200 mg / kg body weight) of the alcoholic extract of fenugreek seeds to normal rabbits produces no significant decrease in blood glucose concentration when given orally; Abdel-Barray *et al.* (43) reported a similar finding. This result disagrees with Vasts *et al.* (44) who observed that the administration of alcoholic extract of fenugreek significantly lower the blood glucose in normal rats.

The effect of alcoholic extract on total cholesterol concentration

Out of the results, there was a significant reduction in the total cholesterol concentration in the diabetic animals treated with fenugreek extract. This finding is in agreement with previous studies (45,46,47). These authors suggested that the inhibitory effect of fenugreek on plasma total cholesterol might be due to the inhibitory effect of this plant on the cholesterol synthesis.

Sharma *et al.* (4) found that the fenugreek contains biologically significant level of saponins. Similar explanation was proposed by many researchers who suggested that saponin would increase fecal excretion of bile acids and subsequent increase of conversion of cholesterol to bile salts and could lower plasma cholesterol concentration (48,49).

Moreover, Sharma (11) proposed that the protein in fenugreek is 26% so it might exert a lipid lowering effect. Also, James, (50) stated that the quality and quantity of protein in the diets have a direct effect on the levels of cholesterol.

Hannana *et al.* (51) showed that hypolipidemic action could also be the result of the retardation of carbohydrate and fat absorption due to the presence of bioactive fiber in the fenugreek. However, Ribes *et al.*, (52) found that the lipid lowering effect of *Trigonella* might also be attributed to its estrogenic constituent, indirect increasing thyroid hormone (T₄). Another author also has shown that this plant stimulates the hepatic lipogenic enzymes, which cause the cholesterol to be decreased (53).

On the other hand, Abou El-Soud *et al.* (54) demonstrated that the two possibilities could be the cause of alteration in lipid profile. Firstly, the rate of lipogenesis is normalized by fenugreek extract in a way similar to the effect of insulin in the lipid metabolism. Secondly, it could be due to the achievement of normoglycemia where there was no further degradation of already accumulated lipid for otherwise glucose starved cells.

The results in the present study showed that there was no significant difference in total cholesterol in normal rabbits treated with fenugreek extract compared with the control group. These results disagreed with the results of Khosla *et al.* (25) who noted that there was decrease in the total cholesterol in normal rats after the administration of fenugreek.

The effect of oral administration of alcoholic extract of *Trigonella foenum-graecum* seeds on total serum protein:

The statistical analysis demonstrates a significant increase in serum total protein concentration in the diabetic treated group with the time of treatment to normal limit. There are no previous studies on the effect of fenugreek seeds on the total serum protein have been reported. These results may reflect the effect of fenugreek seeds extract by stimulating glucose dependent insulin secretion from the pancreatic beta cells (41). This increase in insulin concentration may lead to decrease protein catabolism, amino acids degradation and increase the protein synthesis (55). Similar explanation was proposed by other researchers who studied the effect of garlic, curcumin and *Abroma augusta* on total serum protein concentration (21,56).

التأثيرات البايوكيميائية لبذور نبات الحلبة في الأرانب الطبيعية والمستحدث فيها الداء السكري

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الخلاصة

أجريت الدراسة الحالية لمعرفة تأثير المستخلص الكحولي لبذور نبات الحلبة - *Trigonella foenum graecum* على كل من سكر الدم والكوليسترول والبروتين الكلي للمصل في الحيوانات السليمة والحيوانات المصابة بالسكري المحدث بواسطة الالوكزان .أربعة وعشرون ذكر بالغ من الأرانب المحلية قسمت عشوائياً بشكل متساو إلى أربع مجاميع (٦ لكل مجموعة) وعلى النحو التالي:

مجموعة السيطرة السالبة (أرانب سليمة جرعت ٢ مل من محلول الملح الفسيولوجي و مجموعة الأرانب السليمة المعالجة بالمستخلص الكحولي لبذور الحلبة (٢٠٠ ملغم / كغم من وزن الجسم) جرعت يومياً. ومجموعة السكري الموجبة (أرانب مصابة بالسكري جرعت ٢ مل من محلول الملح الفسيولوجي). و مجموعة السكري المعالجة بالمستخلص الكحولي لبذور الحلبة (٢٠٠ ملغم / كغم من وزن الجسم) جرعت يومياً وعن طريق الفم ولمدة ٦ أسابيع. تم أحداث السكري تجريبياً بواسطة حقن (١٠٠ ملغم / كغم من وزن الجسم) من مادة الالوكزان أحادي التميؤ عن طريق الوريد وبجرعة واحدة. وأجريت الفحوصات التالية أسبوعياً ولكل المجاميع : قياس مستوى السكر في المصل و مستوى الكوليسترول الكلي وبروتين المصل الكلي وقد أظهرت النتائج أن للمستخلص الكحولي لبذور الحلبة قد ادى الى انخفاض معنوي ($P<0.05$) لكل من مستوى السكر والكوليسترول الكلي في المصل لمجموعة المعالجة بمستخلص الحلبة مقابل بمجموعة السكري الموجبة، كذلك أظهرت الدراسة عدم وجود انخفاض معنوي ($P<0.05$) في مستوى سكر الدم في الأرانب السليمة المعالجة بالمستخلص الكحولي مقارنة مع مجموعة السيطرة السالبة. ومن ناحية اخرى أظهرت النتائج زيادة معنوية في مستوى بروتين المصل الكلي لمجموعة السكري الم عالجة بمستخلص الحلبة مقارنة بمجموعة السكري الموجبة.

نستج من هذه الدراسة أن المستخلص الكحولي لبذور نبات الحلبة له تأثير فعال ومؤثر في خفض سكر مصل الدم بالإضافة إلى خفض مستوى الكوليسترول الكلي في مصل الدم في الحيوانات المصابة بالسكري.

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