

Study the effect of *Trigonella Foenum-Graecum* Foenugreek on some parameters of sperm and serum in Alloxan diabetic mice

دراسة تأثير مستخلص نبات الحلبة على بعض قياسات الحيامن والمصل في الفئران
المستحدثة داء السكري بالالوكزان

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Abstract

The influence of fenugreek injected (IP) on male fertility by using following tests (measurement of testosterone level, sperm viability, activity, motility and abnormalities) and on GOT, GPT, Al.ph. and Lipid profile status was studied in normal and alloxan diabetic mice. Alloxan diabetic mice were injected with Trigonella extract for 5 weeks at a dosage of 2g/kg body weight. The Alloxan diabetic mice exhibited enhanced decrease significantly in glucose level in serum. Increased testosterone level and decreased dead sperms, abnormalities and increase motility, decrease in GOT, GPT, AL.PH. and lipid profile, in alloxan diabetic mice.

المستخلص

دراسة تأثير حقن مستخلص نبات الحلبة (بالخلب البريتوني) على خصوبة ذكور الفئران من خلال قياس (مستوى هرمون التستوستيرون في مصل الدم ، فعالية الحيامن ، تشوهات الحيامن) وقياس مستوى انزيم ال GOT, GPT , AL.PH. وتحليل الدهون في الفئران المستحدثة السكري باستخدام الالوكزان والفئران الطبيعية . تم حقن الفئران المستحدثة السكري بمستخلص نبات الحلبة لمدة 5 اسابيع وبجرعة 2 غم/كغم من وزن الجسم ، وقد لوحظ انخفاض مستوى الكلوكوز في مصل الدم في الفئران المستحدثة السكري ، وارتفاع مستوى هرمون التستوستيرون في مصل الدم وقلة عدد الحيامن الميتة والتشوهات ، وزيادة فعالية الحيامن ، كما لوحظ انخفاض ممستوى الانزيمات GOT, GPT, Al.ph. وتحليل الدهون في الفئران المستحدثة السكري باستخدام الالوكزان .

Introduction:

Trigonella fraenum graecum (fenugreek) is traditionally used to treat disorders such as diabetes, high cholesterol, wounds, inflammation, and gastrointestinal ailments [17].

Recent studies suggest that fenugreek and its active constituents may possess anticariogenic potential [15,20].

Trigonella seeds and some of its fraction have a hypoglycaemic effect in experimentally induced diabetes [17, 21] and have hypercholesterolemic activity in rats [10,12]. Fenugreek has very important roles to improve fertility due to it contain diogesine that considered

Materials and methods

Plant materials: Fenugreek seeds were purchased from the local market and identified in a Biotechnology Research Centre-Al-Nahrain University. The seeds were cleaned and finely powdered, the powder was mixed with normal saline (1% w/v) and injected the mice (IP) at level 2g/kg body weight.

Animal care: Healthy adult albino males of Swiss albino strain were obtained from animal house of Biotechnology Research Center- Al-Nahrain University. 35 mice were used in this study, the age of the mice were in the range of 2.5 to 3

precursor for synthesis of sex hormone [22].

Fenugreek display hypoglycaemic and hypo cholesterol acmic effects and are considered tope potentially useful for glucose control and in the treatment of hyperlipidemia and a atherosclerosis in diabetic subjects [20].

The present study, determined the effect of fenugreek in normal and alloxan-diabetic mice on male fertility (sperm viability and abnormalities) , GOT, GPT, alkaline phosphatase and on lipid profile (cholesterol, HDL-cholesterol, Triglyceride).

months old , and the weight in the range 25-30 grams. The animals were housed in small plastic cages, which were cleaned weekly by washing with soap and tap water and sterilized with 70% ethyl alcohol throughout the period of the study. The room temperature was maintained at (24±2) °C, and the animals were exposed to 14 hours light program. **Induction of Diabetes:** Diabetes was induced by a single intraperitoneal injection of alloxan monohydrated (5% w/v) in physiological saline at a dose of 150 mg/kg body weight in a volume of

0.1ml. The diabetic state was confirmed 48 hours after alloxan injection by weight loss [5], and hyperglycaemia [18]. There was 75% mortality in animals treated with alloxan. Surviving mice with a fasting blood glucose level higher than 200mg/dl were included in the study. Seven groups consisting of five animals for each group were maintained as follows:

Experimental group

Control group: Normal mice injected with 0.1ml of physiological saline.

Group A:-Normal mice injected with alloxan 0.1ml to formed diabetic mice.

Group B:-Diabetic mice treated with 0.1ml of Trigonella extract after one week from treated with alloxan.

Group C:- 2 week

Group D:- 3 week

Group E:- 4 week

Group F:- 5 week

General procedure:- Blood sugar levels were determined periodically by heart puncture at the end of (1,2,3,4 and 5 weeks) from treated with alloxan, the mice fasted over night and killed by cervical dislocation.

Treatments of male: - The testes were removed and placed in a sterile disposable Petri dish containing 1ml

TCM-199 medium, then the epididymes were isolated and spermatozoa were obtained from the two tails of epididymes by mixing in 1ml TCM-199, and maintained at 37°C in 5% CO₂ incubator prior treatments.

Microscopically examination:- Spermatozoa were assessed according to WHO Laboratory manual for viability, activity, Motility and abnormalities.

Testosterone assay:- Bio merieux Italia S.P. a vidia campigliano, 58 50015-point A EMA (F₁) Italia miniVIDAS. Was used for the hormonal assay.

C. Bio merieux Sa.69230 marcy l'Etoile – France, testo sterone for 30 sample (test), code No. 09345B. In testosterone test the assay principle combines an enzyme immuno assay sandwich method with a final fluorescent detection (ELFA).

Biochemical measurements:-

1. GOT, GPT.-

According to [16], blood was collected from the mice by heart puncture .The serum was separated by centrifugation at 2000 rpm for 10 min. Then, the serum was taken and treated as follows:

Two test tubes were used for each sample, the 1st one contained the blank reagent and 2nd contains the sample.

These samples were treated as in the following:-

	GPT	GOT
Reagent 1	1 ml	—
Reagent 2	—	1 ml
Incubate for 5 min at 37°C.		
Serum	0.2 ml	0.2 ml
Mix and incubate at 37°C	— 1 hour	— 30 min.
Reagent 3	1 ml	1 ml
Mix. Let stand for 20min at room temp		
NaOH 0.4 N	10 ml	10 ml
Mixed wait 5 min. measure under condition identical to those used for the standard curve.		

Wavelength: 505 nm (490 - 520nm)

Activities of these two enzymes in the serum were estimated from the activity table attached with kit of each enzyme.

3. ALP(alkaline phosphates)

Sample used in this test was the same of serum sample used for GPT& GOT tests.

To estimate the activity of the ALP enzymes, procedure of [11] was used: four test tubes for each sample were prepared, the 1st one contain the sample, the 2nd is the blank sample, the 3rd contain the standard sample and the 4th is the blank reagent, as shown below:-

	Serum sample	Serum bank	Standard	Reagent blank
Reagent	2 ml	2ml	2ml	2ml
Incubate for 5 minutes at 37°C.				
Serum	50µl	—	—	—
Reagent 2	—	—	50µl	—
Incubate for exactly 15 min at 37°C.				
Reagent 3	0.5ml	0.5ml	0.5ml	0.5ml
Mixed well or preferably vortex.				
Reagent 4	0.5ml	0.5ml	0.5ml	0.5ml
Serum	—	50µl	—	—
Distilled water	—	—	—	50µl
Mix. Let stand for 10 minutes in the dark.				
Measure.				

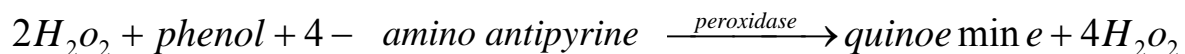
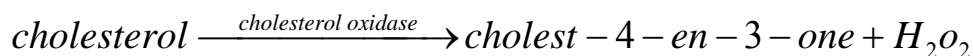
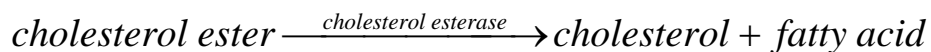
$$\text{Calculation} = \frac{\text{OD serum sample} - \text{OD serum blank}}{\text{OD standard}} \times n$$

4. Lipid profile

Determination of Serum Total Cholesterol in the serum was measured by enzymatic method, with the biomerux kit.

Principle

The principle of this method was lysis of the cholesterol ester to produce cholesterol and fatty acids, then oxidized to produce the quinoemine:



Reagents:	4- 4 samino antipyrine	0.5 mmol/L	
The reagent used in test is a mixture of:	5- peroxidase	≥1000 u/L	
1- phosphate buffer	0.1 mol /L	6- cholesterol oxidase	≥ 200 u/L
2- phenol	15 mol /L	7- cholesterol esterase	≥125 u/L
3- sodium cholate surfaetante	3.74 mmol/L		

Procedure:

The procedure for this method is as follow:

	Reagent blank	Standard	Sample
Standard 200 mg/dL	-	10 µL	-
Sample	-	-	10 µL
Working reagent	1 ml	1 ml	1 ml

After addition, mixing the content of every tube. Allow staying at room temperature for 10 minutes or incubating at 37° C for 5 minuts and reading

absorbance by spectrophotometer at 500 nm. The intensity of the produces color is directly proportional to total cholesterol concentration in the sample.

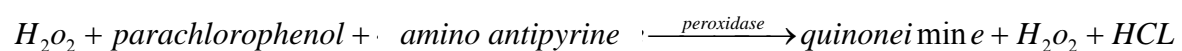
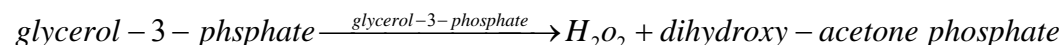
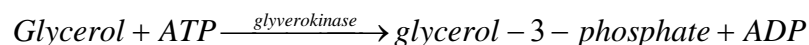
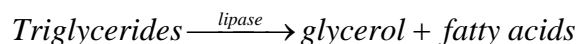
$$\text{Total cholesterol (mmol/L)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 5.17$$

Determiration of Serum Triglyceride

Total triglycerides in the serum were measured by enzymatic with the (biomerieux kit.)

Principle

Total triglyceride determination depend on formation of quinonemine by using a group of enzymes as follows:



Reagents:	5- Lipase	≥ 1000 u/L
The reagent used in this test is a mixture of:	6- Glycerokinase	≥ 200 u/L
	7- Glycerol – 3- phosphate oxidase	≥ 2000 u/L
1- Buffer pH 7.6	100 mmol/L	
2- p – Cholesterol	2.7 mmol/L	8- Peroxidase ≥ 200 u/L
3- Magnesium	4 mmol/L	9- ATP 0.8 mmol /L
4- 4- Aminoantipyrine	0.4 mmol/L	10- Glycerol 2.29 mmol/L

Procedure:

	Reagent blank	Standard	Sample
Standard 200 mg/dl	-	10 μ L	-
Sample	-	-	10 μ L
Working reagent	1 ml	1 ml	1 ml

Gently mix the content of every tube after addition, allow staying at 20 – 25°C temperature for 10 minute or incubating at 37°C for 5 minutes and reading

spectrophotometrically at 505 nm. The intensity of the produced color is proportional to total triglyceride in the sample.

Calculation:

$$\text{Sample concentration} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times n \quad (n = \text{concentration of standard } n = 2.29)$$

Determination of Serum High Density Lipoprotein – Cholesterol (HDL – C):

HDL – Cholesterol the serum were measured by enzymatic method using biomeriex kit,.

2.6.3.2 Reagents:

HDL – cholesterol precipitant	Phosphotungstic acid	40 g/L
	MgCl ₂ . 6H ₂ O	100 g/L
	pH 6.2	1 g/L

Procedure:

	Reagent blank	Standard	Sample
Distilled water	50 µL	-	-
HDL – calibrator	-	50 µL	-
Supernatant	-	-	-
Working reagent	1 ml	1 ml	1 ml

The working solution is the cholesterol enzymatic solution gently mix with the contents of every tube; after the addition let it stay at 20 – 25 for 10 minutes or Calculation:

incubate it for 5 minutes at 37°C and then read spectrophotometrically at 500 nm.

$$\text{HDL. Cho. (mmol/L)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 1.42$$

(1.42 = the concentration of standard)

*Statistical analysis

Statistical analysis was performed to compare two different groups by using ANOVA-test. Statistical significance was determined at $P < 0.05$ [1].

Results and discussions:-

The glucose level increased in serum of normal mice from that indicated to induced alloxan-diabetic mice as reported by [4]. Table (1).

Alloxan diabetic mice treated with Trigonella were shown reduced glucose level in serum from 181.32 ± 1.60 to 274.0 ± 2.04 Table(1). They also conclude that fenugreek seeds improve insulin sensitivity and decrease insulin resistance in diabetic mice [8]. The hypoglycaemia effects have been

attributed to several mechanisms [7]. The amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in pancreatic islet cells [19]. This amino acid appeared to act only on pancreatic beta cells, fenugreek reduced the area under the plasma glucose curve and increased the number of insulin receptors [14].

In humans, fenugreek seeds exert hypoglycaemia effect by stimulating glucose-dependent insulin secretion

from pancreatic beta cells [2] as well as by inhabiting the activities of alpha-amylase and sucrose to intestinal enzymes involved in carbohydrate metabolism [3].

The results obtained increased body weight in alloxan diabetic mice treated with the fenugreek extract Table(2). The increased in body weight due to the fenugreek extract contain materials have very important roles in metabolism such as amino acids and alkaloids [24].

While the increased testes weight due to fenugreek extract contain coumarin which cause accumulation of water in testes . Table(2).

Testosterone level no significant decrease in alloxine male diabetic mice to compared with control male (1.63 ± 0.03 , 1.66 ± 0.91) respectively. Fenugreek extract also increased the testosterone level (2.97 ± 0.03) after 5 weeks from treated. This increased due to that the fenugreek contain diosgenin which have important roles in sex hormone synthesis. [6 , 23]. Table(3).

The results obtained no significant decrease in viability , dead spermatozoa , abnormalities (60 ± 3.23 , 34 ± 1.91 and 25 ± 2.20) respectively in alloxan diabetic male mice to compared with control

male (75 ± 2.66 , 32 ± 0.92 and 22 ± 1.61) respectively. While fenugreek extract increased Table (4).

The increase fertility of spermatozoa due to increase the level of testosterone in serum [6,23].

Table 5 showed increased in triglyceride total cholesterol and HDL-cholesterol 203.40 ± 0.78 , 242.31 ± 1.34 and 218.80 ± 1.72 in alloxan-diabetic mice 166.40 ± 2.70 , 201.30 ± 1.61 and 170.33 ± 2.50 respectively to compared with control 169.51 ± 3.41 , 204.10 ± 2.91 and 176.31 ± 3.02 respectively. Fenugreek seeds also lower cerium TG, chol. and HDL-Chol. 237.3 ± 141 , 87.77 ± 3.01 and 90.60 ± 4.51 respectively after 5 weeks. These effects may be due to saponins ,alkaloids, or to the high fiber content of the seeds.[7, 9]. This study have ability of fenugreek to significantly reduced of GOT, GPT, Al.ph., in alloxan mice from 196.22 ± 2.11 , 68.77 ± 1.94 and 66.42 ± 0.91 respectively to $203.41 \pm 69.12 \pm 191$ and 68.31 ± 1.61 respectively after 5 weeks from treated with fenugreek seeds. Table(6) The method of action is unknown but may be due to the saponine, alkaloids, or to the high fibre content of the seeds.[13]

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