رقم البحث (11)

EFFECT OF TRIGONELLA FOENUM-GRAECUM (FENUGREEK) SEEDS POWDER ON RENAL NEPHROPATHY AND HYPERLIPIDEMIC COMPLICATIONS IN ALLOXAN INDUCED DIABETIC RATS

BY

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ABSTRACT

This work was carried out to evaluate the short-term effect of oral administration of a Trigonella foenum-graecum seeds powder (TSP) on blood glucose level, Renal nephropathy and Hyperlipidemic complications and compared with Metformin in alloxan induced diabetic rats. Diabetes was induced in albino rats by administration of a single dose of alloxan monohydrate 5% (125 mg/kg, i.p.) Eighty mature male albino rats weighted 150-180 gm. Were distributed randomly into four groups (20 ineach). The first group (G1) was used as a control. diabetic control was injected i.p. with Alloxan (G2). (G3) was Alloxan induced diabetic rats treated with FSP in a dose of 5% powdered . (G4) was Alloxan induced diabetic rats treated with Metformin HCL in a dose of 0.5 gm/kg/day soluble in Distilled water. Serum samples were collected After 21 days from the beginning of glucosuria following overnight fasting for determination of Oral Glucose Tolerance Curve (OGTC), lipid profile, Creatinine and Blood Urea. Kidney were obtained after sacrificed at the end of the experiment for histopathological study. The results indicated that oral administration of FSP or Metformin HCL significantly enhanced the glucose lowering ability and improvement in lipid profile and kidney functions as compared with (G2). Accordingly, it could be recommended that oral administration of FSP is effective in controlling of Hyperglycemia, Renal nephropathy and Hyperlipidemic complications in diabetes mellitus.

INTRODUCTION

Diabetes mellitus is one of the major metabolic disorders, affecting a large proportion all over the world **(Ziment et al., 2001)**. According to recent estimates approximately 215 million people all over the world suffer from diabetes mellitus and 80 – 90 % of them are from type II diabetes **(Bennett, 1997). Gabir et al. (2000) and Grover et al. (2001)** reported that nephropathy is one of the diabetic complications**.** Moreover Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes **(Bierman EL et al., 1975).** In addition, other studies (**Sochor et al., 1985**) reported that most of the metabolic complications associated with type-1 diabetes are due to insulin deficiency and related to glucose under-utilization of the insulin-dependent tissue, such as liver, and glucose over-utilization of the insulin-independent tissue, such as kidney**.**

In any form of management of diabetes with insulin or drug, diet is a common factor. With respect to diet, plants and foods of medicinal value have proved to be very useful and are in wide usage as they combine two basic central factors, food and medication **(Grower et al., 2002).** Fenugreek seeds are known to improve diabetes status **(Billard et al., 2001).** Medicinal properties of fenugreek such as hypocholesterolemic and hypolipidemic have also been studied **(Genesh Bhat et al., 1985).** Moreover**,**Trigonella foenum-graecum (Fenugreek) exerts its hypoglycemic effect by delaying glucose absorption and enhancing its utilization **(Raghuram et al 1994).**

Metformin-HCl is regarded as the first choice for therapy of type 2 diabetes by reducing blood glucose without inducing hypoglycemia and further hyperinsulinaemia, as well as reducing feed intake, metformin-HCl provides a suitable antidiabetic treatment **(Ashwell1 and Mc Murtry, 2003) .**

Alloxan is widely used to induce experimental diabetes, selectively destroys the insulin-producing pancreatic B- cells without affecting other islet cells and is associated with marked reduction in islet cell Super Oxide Dismutase (SOD) activity(**Halliwell and Gutteridge, 1989**.).

The objective of this study is to investigate the effect of Trigonella foenum-graecum (Fenugreek) on Alloxan induced Diabetes Mellitus and its nephropathy and hyperlipidemic complications when applied separately and in comparison with Metformin in rats.

MATERIALS AND METHODS

1- Experimental animals:

Eighty Mature male albino Wistar rats of age range (2.5-3.0 months) and weighted about 150 – 180 gm obtained from experimental unit, Faculty of medicine, Assute university. Rats were maintained in the animal house at a constant temperature (25°C) and relative humidity 55%. The animals were maintained 0n adequate stable commercial balanced diet and given tap water ad libitum until treatment or time of sacrifice.

2- Chemical: Alloxan monohydrate were obtained from Sigma Chemical Co. (St. Louis, Mo U.S.A) and used in a dose of 125 mg/kg, i.p. freshly prepared in 0.9 N saline as a single dose intraperitoneally injected. ***(N. A. Trivedi et al., 2004)***

3- Drugs (Hypoglycemic agents):

I. Plant (Alternative hypoglycemic agents) Trigonella foenum-graecum (Fenugreek) seeds powder (FSP) were purchased from Upper Egypt local market and used as 5% cleaned, dried and finely powdered (i.e. 5gm of dry FSP in 95gm girding rat feed). (***Farmington Hills, Michigan (2002)***

II. Metformin HCL (Hypoglycemic drug) Cidofage (produced by Chemical industries Development Co. Giza .A.R.E) and used in a dose of 0.5 gm/kg/day soluble in Distilled water ***(Trivedi et al., 2004)***.

4- Experimental groups:

Eighty mature male albino rats weighted 150-180 gm. Were distributed randomly into four groups (twenty/ group). Group (1) was left without treatment and used as a control. Group (2) was injected i.p. with Alloxan (diabetic control). Group (3) Alloxan induced diabetic rats treated with FSP in a dose of 5% powdered (i.e. 5gm of dry FSP in 95gm girding rat feed). Group (4) Alloxan induced diabetic rats treated with Metformin HCL (Cidofage retard 850 mg/Tablet) in a dose of 0.5 gm/kg/day soluble in Distilled water.

5- Induction of diabetes and grouping of experimental animals:

Rats were starved for 24 h and divided into control and experimental groups, for the induction of diabetes, each rat in experimental group was injected alloxan monohydrate 125 mg/kg, i.p. freshly prepared in 0.9 N saline. The detection of diabetes induction was checked in alloxan treated rats using urine glucose detection strips.

6- Sampling:

After 21 days from the beginning of glucosuria blood samples were collected from medial cunthus of the eye following overnight fasting before and 30, 60, and 120 min postprandial of glucose in a dose of 100 mg/kg B.W. and sera were separated immediately for biochemical changes. Kidney were obtained after sacrificed at the end of the experiment and preserved in buffer saline formalin for histopathological study.

7- Biochemical analysis:

Oral glucose tolerance curve (fasting, 30, 60 and 120 min post prandial) by using commercial kit supplied by ***(Human, Germany)***, blood urea nitrogen ***(Putton and Crouched, 1977)*** and serum creatinine ***(Houto, O., 1985)***, Total cholesterol ***(Mellattini, 1978)***, Triglycerides ***(Bucolo and David, 1973)***, HDL ***(Clark et al., 1983)*** and LDL ***(Friedwald et al., 1972)***.

8- Histopathological finding:

For histopathological examination the kidneys were fixed in 10% neutral buffered formalin and were processed for routine histopahological examination.

9- Statistical analysis:-

The results were reported as the mean + S.E. Statistical significance was determined using analysis of variance a of variance according to ***(SAS, 1996)***.

RESULTS AND DISCUSSION

Chronic complications of diabetes are more evident in type 2 diabetes because of its insidious onset and accidental discovery in most of its situations.

Although the presence of a lot of different research trials trying to discover new lines of treatments of diabetes, the door is fully opened in front of the natural herbal preparations which are considered safe, economic and effective preparations. The strict control of diabetes is considered the most effective line of treatment in prevention of complications.

For Evaluation of FSP treatment of Renal nephropathy and Hyperlipidemic it was compared to a drug Metformin which used as a gaid in this study.

The effect of Trigonella foenum-graecum on serum glucose level :

Table (1) elucidate the OGT Curve showed that oral administration of (FSP) to the diabetic rats (G3) induce significant decrease in serum glucose level which reaches near the fasting level after 2 hours. This descending limb represents glucose utilization. Such result were coincided with G4 (diabetic treated with Metformin). This reduction was explained by ***Raghuram et al. (1994)*** who reported that Trigonella foenum-graecum (Fenugreek) exerts its hypoglycemic effect by delaying glucose absorption and enhancing its utilization. Moreover ***Fransworth and.Marles (1995)*** concluded that the hypoglycemic alkaloid trigonelline from FSP is a plant growth inhibitor due to inhibition of insulinase enzyme.

Effect of Trigonella foenum-graecum on lipid profile :

The Values of serum cholesterol, Triglycerides and LDL presented in Table (2) showed significant reduction in its levels in G3 when compared with G2. Also the raised level of VLDL in G2 returned near to the control level in G3 this could be attributed to the good control of blood glucose as described by ***Austin et al., (1988***) who noted that VLDL particles are commonly seen when there is poor glycemic. furthermore HDL was significantly increased. These results were nearly correlated with those of G4. Accordingly it could be concluded that the hypolipidemic effect of Fenugreek or Metformin might conceivable to be due to inhibition of cholesterol absorption and reduce plasma cholesterol levels in experimental animals, and are therefore, of potential pharmacological utility in the treatment of hypercholesterolemia(***Yue Zhong Shu.,1998)***.

Trigohnella foenum-graecum and renal complications:

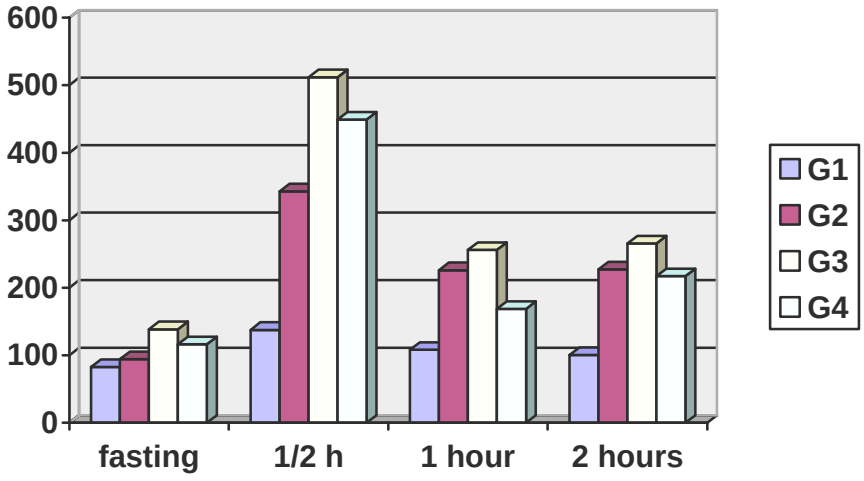
The elevated values of serum creatinine and blood urea in G2 due to diabetic nephropathy were returned to the normal values in G3 and G4. These results were confirmed by the histopathological study of the kidney where in diabetic rats kidney showed Cloudy swelling, and Focal segmental glomerulosclerosis a result which coincide with that reported by ***Barbosa et al., 1994, Mundel and Shankland, (2002)*** who showed that the structural changes of the glomerular basement membrane and the loss of glomerular podocytes by the kidney are considered the most important mechanism involved in the genesis of diabetic nephropathy. As podocytes are unable to regenerate, this produce nude area on the

glomerular basement membrane which leads to progressive sclerosis of the glomerulus. ***Meyer et al (1999)***. In the glow of previous notion one could attribute this improvement in kidney functions after treatment with FFS or MET to the reduction of sclerosis of the glomerulus due to improvement of blood glucose and this effect is shown in histopathological examination of kidney in G3,G4. In contrary, ***Michels et al. (1981)*** reported that Although both of streptozotocin and alloxan have been used as diabetogenic chemicals, there is a possibility that these chemicals affect glomerular cells as the direct toxins against kidney cells.

Table (1): The effect of FSP or MET on oral glucose tolerance (OGT) Curve in the different groups.

PARAMETER	CONTROL	DIABETIC	FSP	MET.
Fasting	82.6 ± 3.50^{c}	137.6 ± 2.66^{a}	108 ± 4.04^{b}	100.4 ± 3.2^{b}
30 min. PP	94.2 ± 20.46^{c}	342.8 ± 3.94^{a}	226 ± 3.32^{b}	227.2 ± 3.85^{b}
60 min. PP	138.6 ± 3.23^{c}	511.94 ± 10.11^{a}	256 ± 4.18^{b}	265.6 ± 3.91^{b}
120 min. PP	116.2 ± 2.56^{d}	449.2 ± 8.83^{a}	168.6 ± 4.2^{c}	217.2 ± 2.73^{b}

Means within the row carry different superscripts $^{a-d}$ are significantly different at level P<0.01.

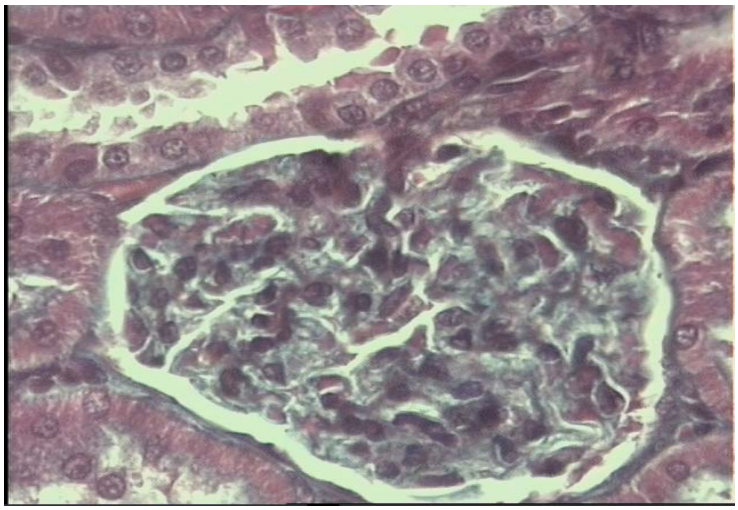


(OGT Curve)

Table (2): The effect of TSP on blood urea and serum creatinine, S.Total Cholesterol, S.Triglycerides, HDL, LDL on the different groups of rats.

PARAMETERS	CONTROL	DIABETIC	FSP	MET.
S. Creatinine	0.84 ± 0.03^{c}	1.2 ± 0.03^{a}	0.93 ± 0.02^{bc}	0.97 ± 0.07^{b}
Bl. Urea	53.38 ± 2.53^{b}	76.2 ± 3.60^{a}	60.4 ± 2.87^{b}	62.7 ± 2.92^{b}
S.T.Cholisterol	83.86 ± 1.93^{b}	102.6 ± 3.37^{a}	83.72 ± 3.07^{b}	79.7 ± 1.55^{b}
S.Triglycerides	97.96 ± 2.77^{c}	137.58 ± 2.93^{a}	111.22 ± 3.42^{b}	105.1 ± 2.6^{bc}
HDL-c	48.74 ± 0.94^{a}	34.16 ± 2.04^{c}	41.28 ± 1.82^{b}	42.56 ± 2.83^{b}
LDL-c	15.5 ± 1.86^{b}	40.88 ± 2.54^{a}	20.2 ± 2.98^{b}	16.1 ± 1.3^{b}
VLDL	19.6 ± 0.56^{c}	27.56 ± 0.58^{a}	22.24 ± 0.67^{b}	21.04 ± 0.52^{bc}

Means within the same row carry different superscripts $^{a-d}$ are significantly different at level $P<0.01$.



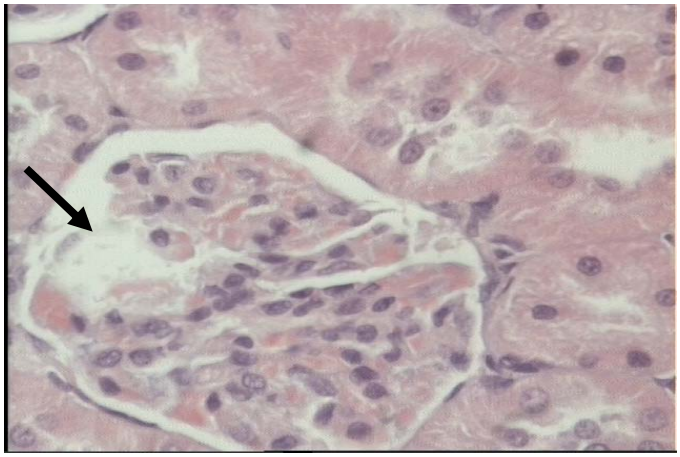
Picture (1) : control group : *400X

Masson's trichome stains (MT) *100X

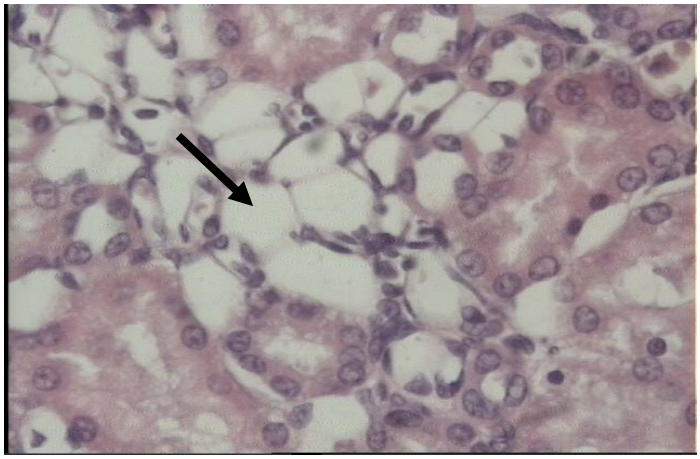
Normal kidney, with intact glumeruli and tubules. No evidence of fibrosis.

(Normal glomerulus. Note the open capillary loops and thinness of their walls.

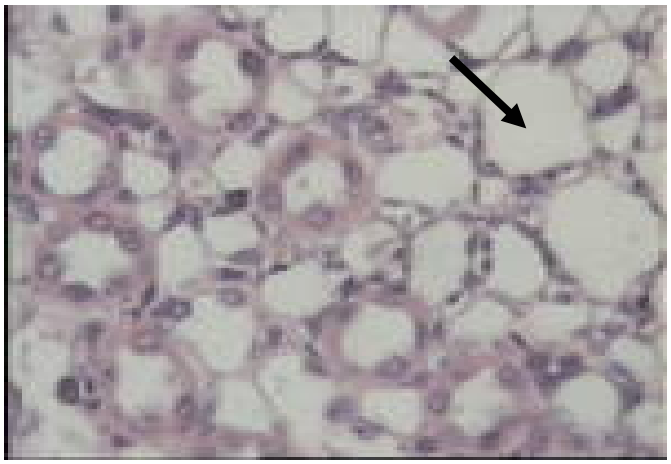
Tubular histopathology: normal tubular histopathology. The tubules are back-to-back. Brush borders can be seen on the luminal borders of cells in the proximal tubule.



Picture (2) : Diabetic group : * 400X
Cloudy swelling proximal tubules show mild swelling hydropic degeneration.
Focal segmental glomerulosclerosis. The portion of the glomerulus arrowed shows loss of capillary loops.



Picture (3) : Diabetic with TSP treatment : * 400X
Focal interstitial fatty change, not associated with inflammation. Focal fatty change.



Picture (3) : Metformin treatment : * 400X

Fatty change, not associated with inflammation.

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الملخص العربي

تأثير مطحون بذور نبات الحلبة على ارتفاع دهون الدم و اعتلال الكلية السكري الناتج عن مرض السكر المستحدث بالألو كسان في الفئران.

أسامه بهجت مصطفى الجزار* أحمد أمير رضوان السيد**

***قسم الكيمياء الحيوية كلية طب بيطري البستان فرع دمنهور جامعة الإسكندرية**

****قسم الكيمياء كلية العلوم جامعة المنيا**

أجريت هذه الدراسة على الفئران المصابة بمرض السكر المستحدث بواسطة جرعة واحدة من الألو كسان بهدف دراسة تأثير إضافة مطحون بذور نبات الحلبة (٥%) على مستوى السكر و الدهون في الدم وكذالك وظائف الكلى. استخدم عدد٨٠ من الفئران البيضاء البالغة والتي تزن ١٥٠ – ١٨٠ جرام، قسمت إلى أربع مجموعات تحتوى كل مجموعة ٢٠ فأر. المجموعة الأولى استخدمت كمجموعة ضابطة، المجموعة الثانية والثالثة والرابعة تم حقنهم بجرعة واحدة من الألو كسان (١٢٥ مجم/كجم) داخل تجويف البطن لاستحداث مرض السكر، استخدمت المجموعة الثانية كمجموعة ضابطة لمرض السكر بدون علاج، تم إضافة مطحون بذور الحلبة لعلائق المجموعة الثالثة، وإضافة دواء الميتفورمين (الجلوكوفاج) (٠,٥ جرام/كجم/اليوم)في مياه الشرب للمجموعة الرابعة كمجموعة تأكيدية استخدم فيها دواء شائع و متداول. أخذت عينات الدم بعد ٢١ يوم من بداية ظهور السكر في البول وذبحت الفئران بعد انتهاء التجربة وأخذت الكلى للدراسة الهستوباثولوجية.

تم دراسة التأثير على منحنى السكر والدهون في السيرم وكذلك معدل التغيرات في اليوريا والكرياتينين.

دلت النتائج على وجود نقص ملحوظ في مستوى السكر و الدهون المرتفعين نتيجة مرض السكر بالدم كما أن معدلات اليوريا والكرياتينين قد عادت إلى المستوى الطبيعي.

وبالفحص الهستوباثولوجى دلت النتائج على وجود تغيرات باثولوجية في كلى الفئران المصابة بمرض السكر تم تحسنها في المجموعات المعالجة بمطحون بذور الحلبة والميتفورمين.

من هذه الدراسة يتبين لنا الدور الهام لاستخدام مطحون بذور نبات الحلبة لعلاج مرض السكر وللتقليل من بعض مضاعفاته مثل ارتفاع في مستوى الدهون بالدم أو الخلل الناتج في وظائف الكلى.