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Hypoglycemic And Hyperinsulinemic Effects Of Ferula Assafoetida On Diabetic Male Albino Rats

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Abstract

In the current study, thirty adult male albino rats were used to investigate the effect of Ferula assafoetida on carbohydrate metabolism in alloxan-induced diabetes. Rats were divided into three equal groups, control, diabetic non treated and diabetic Ferula assafoetida treated groups. After thirty days of treatment five rats of each group were sacrificed and the others were left without any additional treatment for another 15 days (recovery period) then were sacrificed. Body weight, blood glucose, serum insulin and liver glycogen content levels were determined for each rat at the end of each period. It was noticed that Ferula assafoetida treatment led to a significant improve in hyperglycemia, hypoinsulinemia, decreased liver glycogen and increased percentage of body weight change caused by alloxan. And this improvement was also seen after the recovery period.

Ferula assafoetida treatment led also to marked improvement in the histopathological degenerative changes in the **β** cells of islets of Langerhans caused by alloxan after both the treated and recovery periods.

Introduction

Diabetes is a common disease, with major global public health consequences ***(Williams and Pickup, 1999).*** The diabetic patients needed alternative therapies to control all of the pathological aspects of diabetes and the high cost and poor availability of current therapies in many rural populations, particularly in developing countries ***(Marles and Farnsworth, 1995).*** The traditional antidiabetic plants might provide this useful source of new oral hypoglycemic compounds.

Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system. A wide variety of the traditional herbal remedies are used by diabetic patients, especially in the third world countries***(Day, 1998 & Gray and Flatt, 1998)*** and may, therefore, represent new avenues in the search for alternative hypoglycemic drugs. The folk medicine in Kuwait has described several kinds of herbs, belonging to various families to be used in the treatment of diabetes mellitus ***(Eskander and Won Jun, 1995).***

Ferula assafoetida Family (Umbellifervae), Devil's drug is native to Iran, Afghanistan, and Pakistan. In the 7th century B. C., Charak Samita, a Hindu medical treatise, proclaimed assafoetida the best remedy for clearing gas and bloating. The asafetida's Oleo-gum-resin are the main parts used, where it contains 6.17% volatile oil, as well as resin and gum. The volatile oil contains disulphides, which have an expectorant action. The oil also settles the digestion. Assafoetida is taken for bronchitis, bronchial asthma, who-ping cough and other chest problems. It also lowers blood pressure ***(Chevallier, 1996)***. Sulfur compounds in the oil may protect against fat-induced hyperlipidemia ***(Duke, 2002).***

So that, this study is a step to evaluate and follow up the effect of Ferula assafoetida alone as a hypoglycemic agents and to show whether it has an insulin like-action or accelerates insulin secretion from pancreatic cells.

Material And Methods

Material:

A-Animals: Thirty adult male albino rats of local strain with body weight (b. wt.) ranging between (120-140 gm) were used in the current work. Rats were divided into three equal groups:

Group I (Control group), were given subcutaneous (s. c.) saline solution (0.01 ml / 100gm b. wt.).

Group II (Diabetic group), were given s.c alloxan (120 mg / kg b. wt.) in order to induce diabetes mellitus ***(Dunn et al., 1943)***.

Group III (Ferula assafoetida treated group), were given alloxan to induce diabetes then given Ferula assafoetida water extract (0.01 g/100 gm b. wt.)Orally once daily for one month.

B-Drugs and chemicals:

Alloxan (powder from B.D.H chemical LTD, England), dissolved in acetate buffer (pH 5.5) prepared immediately before use.

Ferula assafoetida resin, brought from the local market for preparing water extract.

Methods:

- Induction of diabetes mellitus: By giving s.c freshly prepared alloxan solution 120 mg / kg after an overnight fasting of the animals then 48 hours later blood glucose level was determined by glocometer. Rats with blood glucose level ranging from 180 to 250 were considered diabetic *(Dunn et al., 1943).*

***- Preparation of water extract of Ferula assafoetida*:** 50 grams of the dry resin of the plant was boiled in 100 ml distilled water for 10 minutes. After cooling to room temperature it was filtered and stored in a refrigerator till the time of use (dose 0.1g/100g b. wt).

- Preparation of serum and determination of various parameters: After thirty days of the experiment, blood was withdrawn from the retrobulber venous plexus of five rats of each group, left to clot then centrifuged to separate serum. The remaining five rats of each group were left for another two weeks without any additional treatment, then serum was prepared as mentioned before. At the end of each period, Body weight, blood glucose were determined ***(Teitz, 1986),*** serum insulin ***(Reeves, 1983)***, livers were taken for determination of liver glycogen ***(Joseph, 1955***). Pancreases were taken, stained with Hematoxylin and Eosin (HX & E) and modified aldhyde fuchsin ***(Halami, 1952)*** for histological study.

Statistical analysis was performed using t-test and all the results were expressed as means $\pm$ standard error. The statistical difference among the control, diabetic and treated group were assessed by the method of ***Snedecor and Cohron (1980).*** The experimental findings were considered statistically significant ($P<0.05$), highly statistically significant ($P<0.01$).

Results

Fig. (1a) shows the effect of Ferula assafoetida treatment on percentage of body weight change, of male rats after both experimental duration. After treatment period a significant increase in body weight ($P<0.01$) was recorded in comparison with both control and diabetic rats, which no significant change was noticed after the recovery period in comparison with control one.

Ferula assafoetida had marked hypoglycemic effect ($P<0.01$) on serum glucose level from this result it was obvious that the liver glycogen content of Ferula assafoetida treatment rats was significantly increased ($P<0.01$) in comparison with diabetic rats. No significant change in there two parameters were recorded in comparison with control rat till the end of the experiment.

Concerning the effect of Ferula assafoetida on serum insulin level of treated rats, the present results revealed marked and significant inhibition ($P<0.01$) in insulin activity in comparison with control group all over the experimental periods.

Histological examination of slides of pancreas stained with Hx & E of control group showed normal pancreatic islets with rich vascular supply while, modified aldhyde fuchsin stain showed the three main types of cells of the pancreatic islets (alpha, beta and delta cells). **β** cells were more abundant, occupy the central portion of the islet and contain numerous granules. Alpha and delta cells occupy the periphery of the islet. Delta cells are usually adjacent to alpha cells and are somewhat larger in size. Alpha cells are granular and polygonal with central spherical nuclei (figs. 5a&b). Alloxan administration led to shrinkage of the normal architecture of the pancreatic islets. The cytoplasm of **β** cells was vacuolated with degenerated nuclei and many necrotic cells were seen (figs. 6a&b). Ferula assafoetida treatment showed normal architecture of the pancreatic islets. The cytoplasm became granulated, the vacuolated **β** cells become more specially after recovery period and nuclei become normal (figs. 7a&b). The improved histological picture caused by Ferula assafoetida treatment was seen after the recovery period too.

Otherwise, the current study indicated insignificant change in the number and diameter of alpha cells and their nuclear diameter in diabetic and Ferula assafoetida treated group as compared to control till the end of the experiment (Figs. 8a,9a&10a). But the islets of diabetic rats showed reduction in pancreatic beta cells number and significant increase in their cellular and nuclear diameter when compared with control group throughout the experimental period. While, Ferula assafoetida extract ameliorated the changes represented by increased number of **β** cells when compared with diabetic rats after treated and recovery periods at the same time recorded no change in **β** cells number and their cellular and nuclear diameter when compared with normal islets during the experimental period but it was still vacuolated (Figs. 8b, 9b & 10b).

Concerning delta cells the present data showed insignificant changes in their number, diameter and nuclear diameter in the diabetic group and recorded a significant increase after recovery period. Otherwise, the treated group recorded a significant decrease throughout the experiment (Figs. 8c, 9c& 10c).

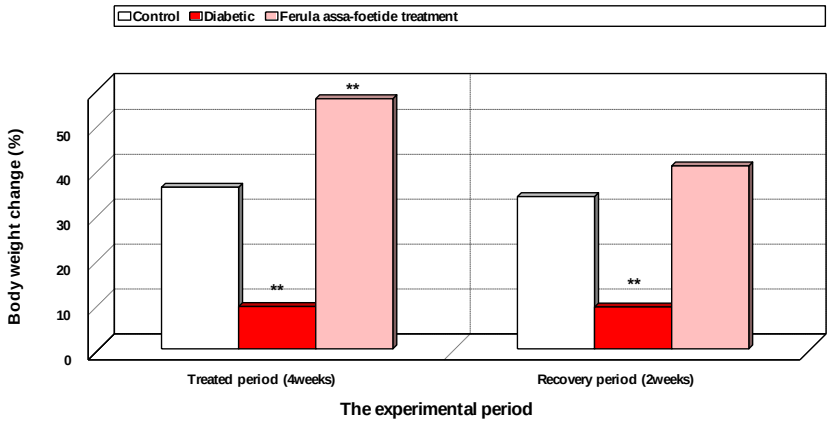


Fig.(1a): Percentage of bodyweight change in control, diabetic and plant extract treated male albino rats after 4 weeks of treatment and 2 weeks of recoveryperiod .

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

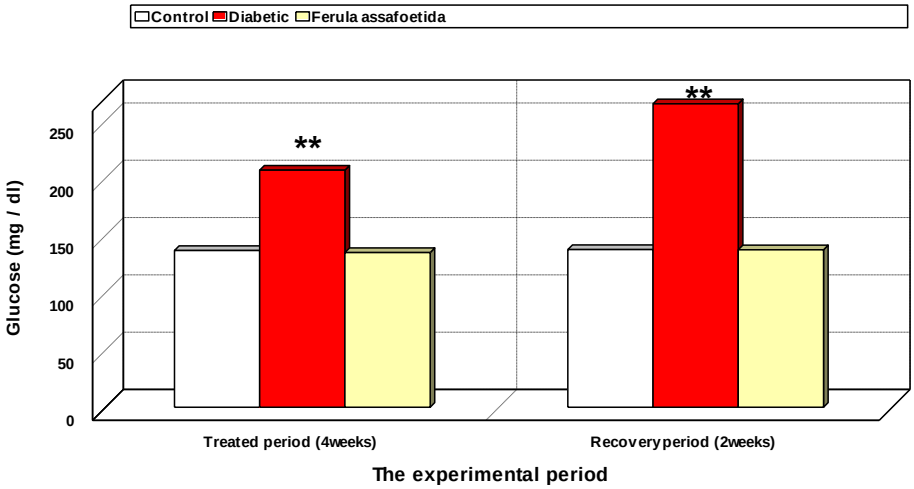


Fig.(1): Serum glucose level in control, diabetic and Ferula assafoetida extract treated male albino rats after 4 weeks of treatment and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

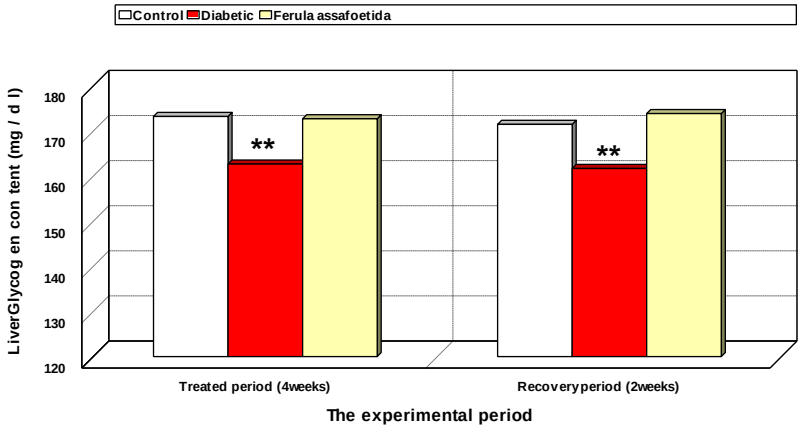


Fig.(2): Glycogen contentof liver in control, diabetic and Ferula assafoetida extracts treated male albino rats after 4 weeks of treatment and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

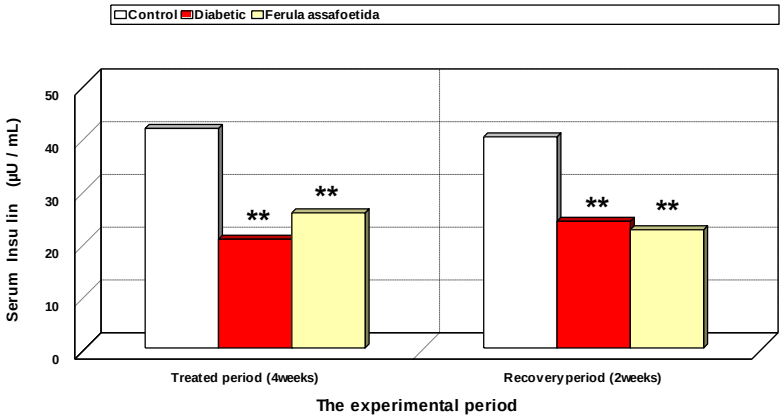


Fig.(4): Serum insulin level in control, diabetic and Ferula assafoetida extract treated male albino rats after 4 weeks of treatment and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

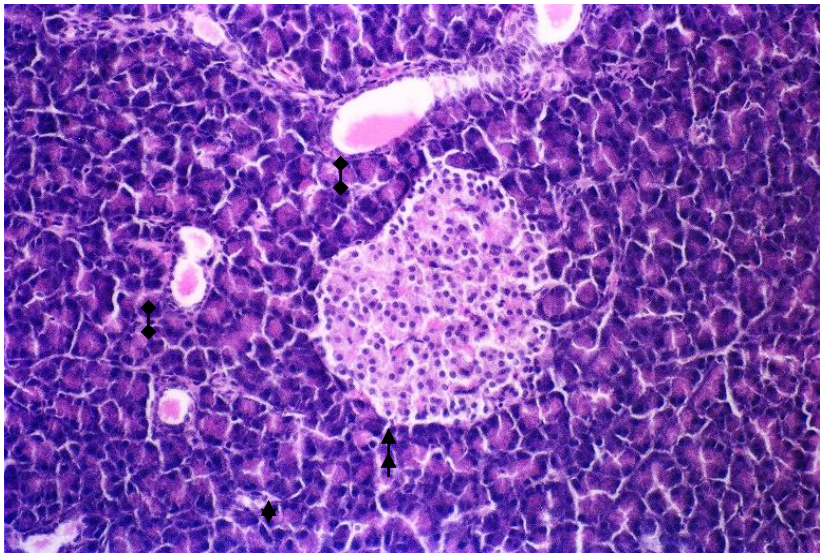


Fig. (5 -a) A photomicrograph of pancreas of control rat stained with HX & E stain shows normal islet cells architecture pancreatic acini and blood vessels (X 400).

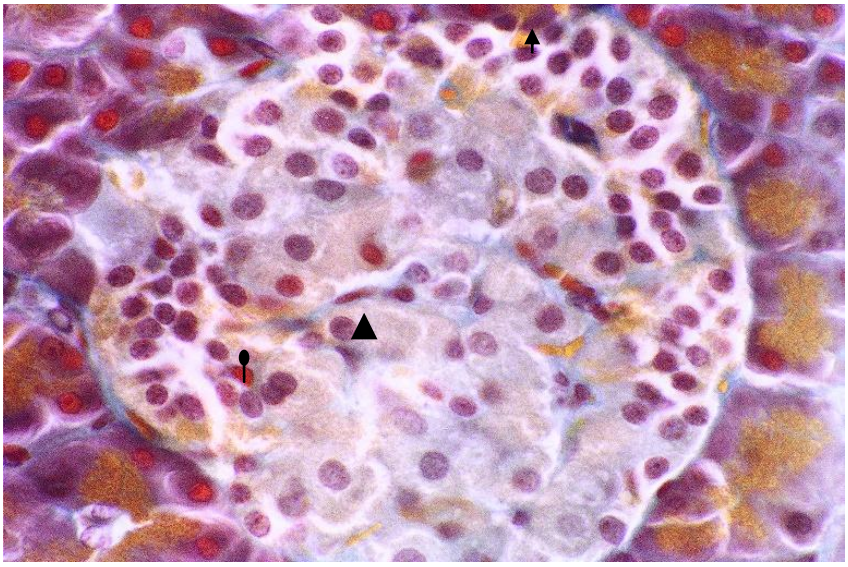


Fig. (5-b) A photomicrograph of pancreas of control rat stained with modified aldhyde fuchsin stain shows normal β cells ( ), delta cell ( ) and alpha cell ( ) of islets of Langerhans (X 1000).

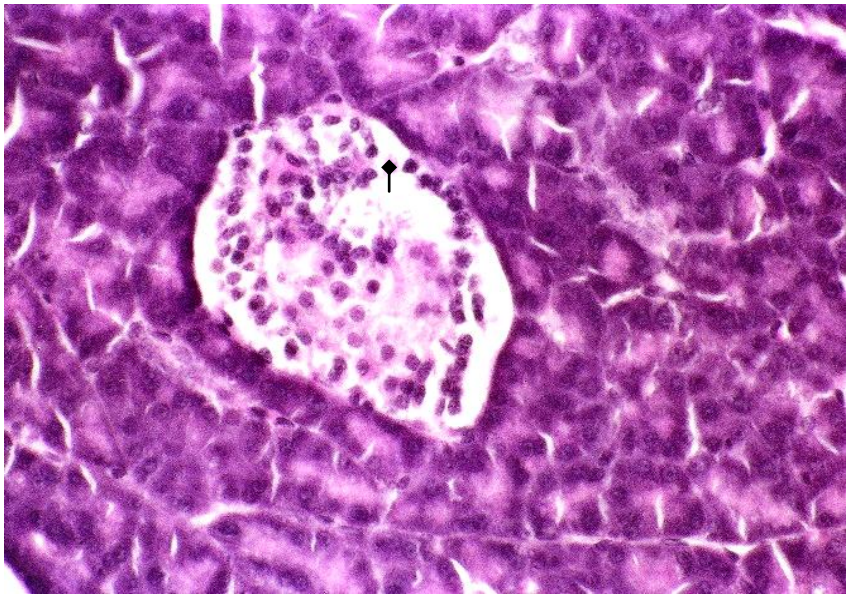


Fig. (6- a)A photomicrograph of pancreas of alloxan-induced diabetic rat stained with HX & E stain shows shrunken islet architecture and necrosis (X 400).

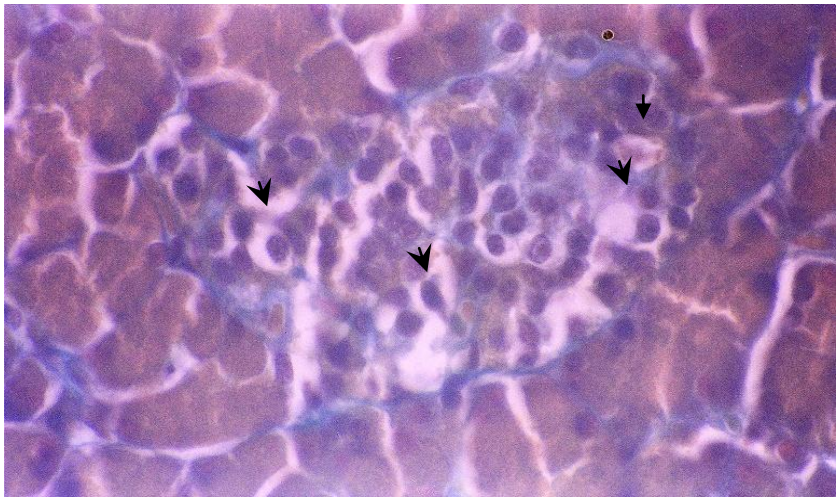


Fig. (6- b) A photomicrograph of pancreas of alloxan-induced diabetic rat stained with modified aldhyde fuchsin stain shows degeneration of β cell with cytoplasmic vacuoles (X 1000).

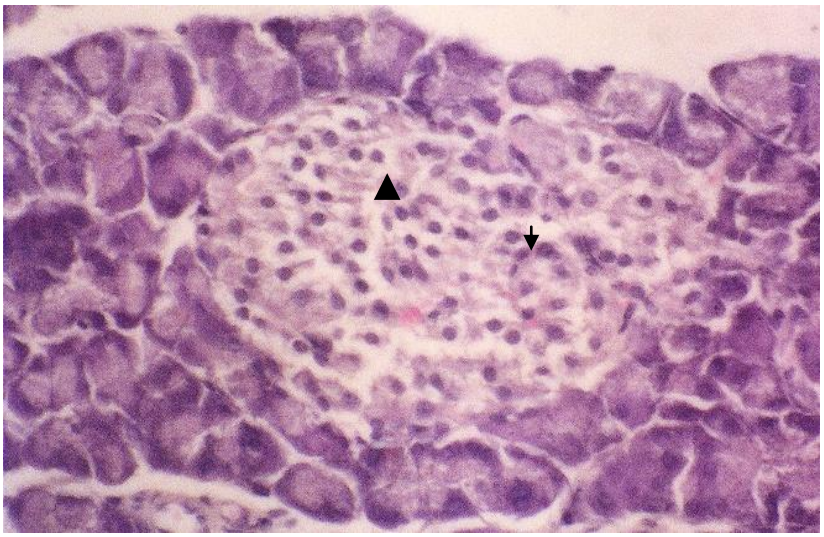


Fig. (7- a) A photomicrograph of pancreas of rat of Ferula assafoetida treated group stained with HX & E shows restoration normal islets architecture β cell and alpha cell (X 400).

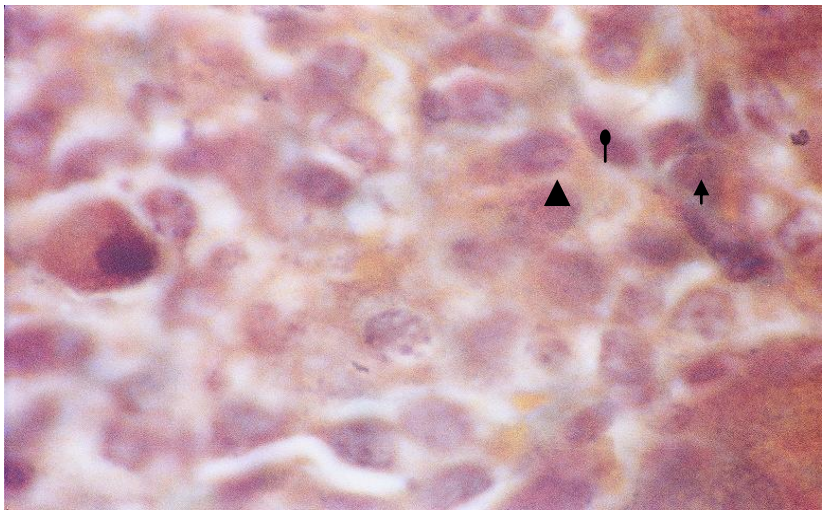
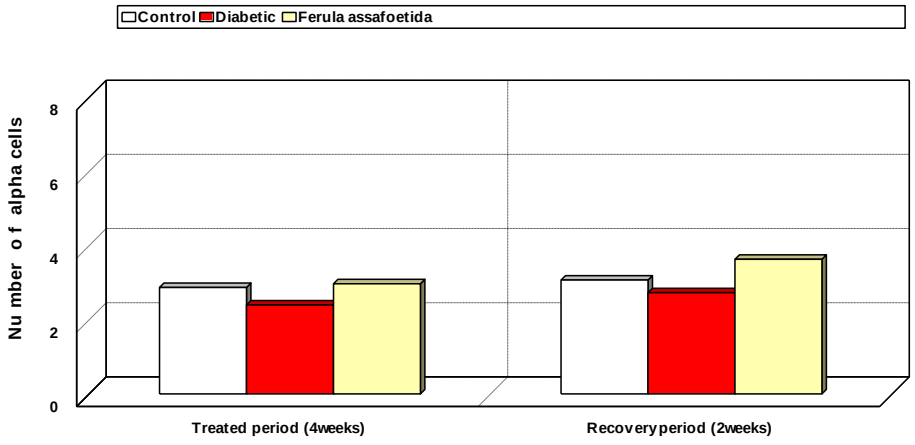
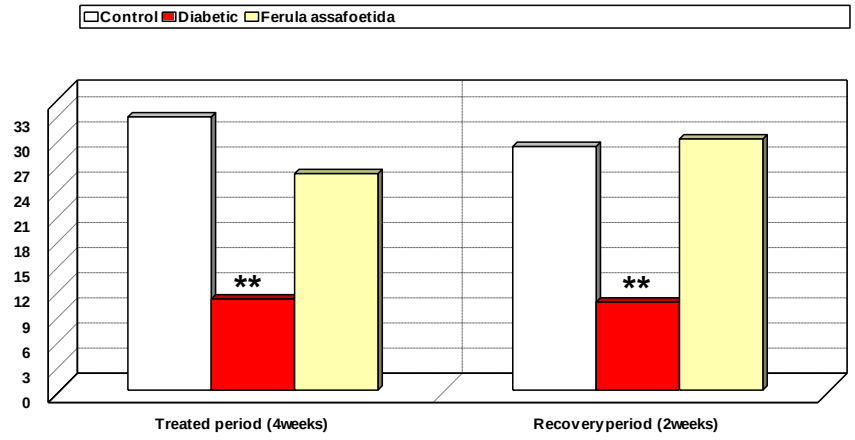


Fig. (7- b) A photomicrograph of pancreas of rat of Ferula assafoetida treated group stained with modified aldhyde fuchsine stain show β cells , delta cell and alpha cell (X 1200).



(Fig.8a): Means of changes in the number of alpha cells in the islet of Langerhans in the control, diabetic and plant extract treated male albino rats after 4 weeks of treat and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)



(Fig.8b): Means ofchanges in the number of beta cells in the islet of Langerhans in control, diabetic and plant extract treated male albino rats after 4 weeks of treatr and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

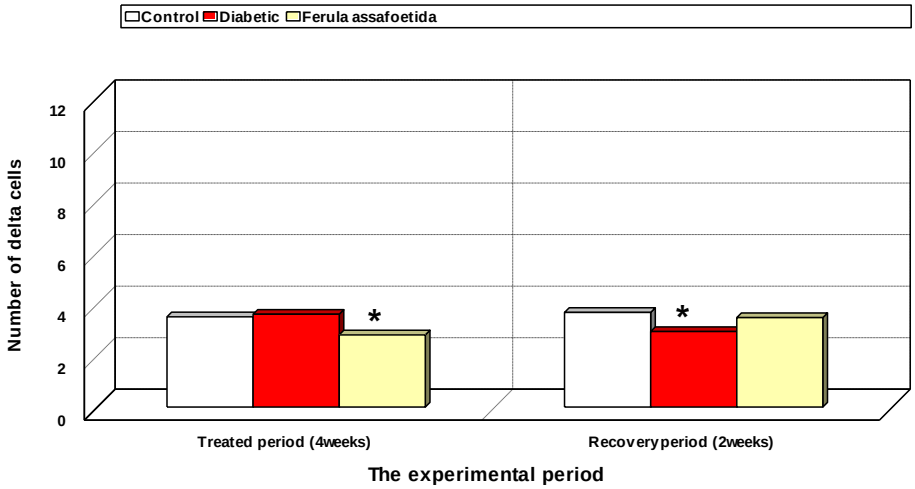


Fig.(8c): Means of number of delta cells in the islet of Langerhans in control, diabetic and plant extract treated male albino rats after 4 weeks of treatment and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

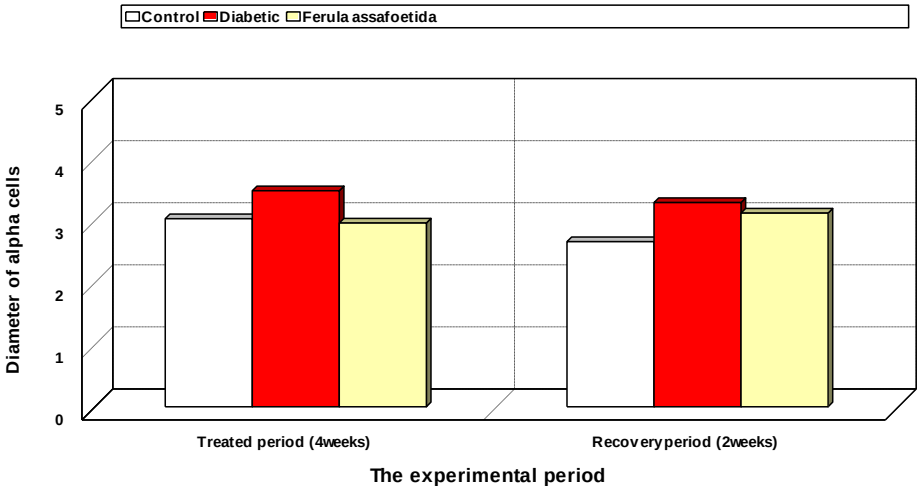


Fig.(9a): changes in the diameter of alpha cells in the islet of Langerhans in control, diabetic and plant extract treated male albino rats after 4 weeks of treatment and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

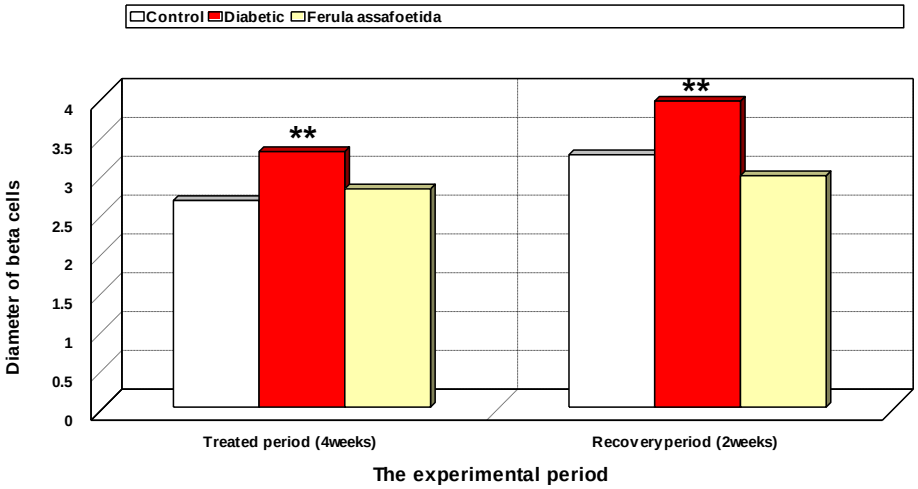


Fig.(9b): changes in the diameter of beta cells in the islet of Langerhans control, diabetic and plant extract treated male albino rats after 4 weeks of treatment a 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

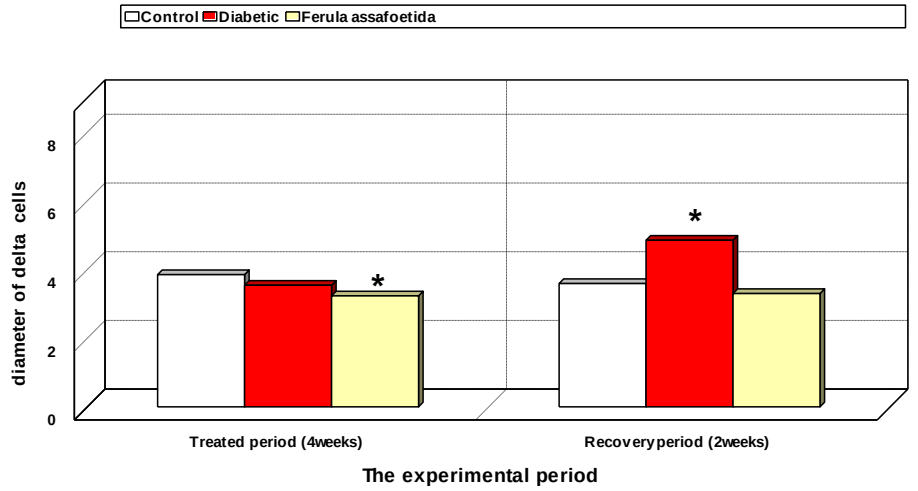


Fig.(9c): changes in the diameter of delta cells in the islet of Langerhans control, diabetic and plant extracts treated male albino rats after 4 weeks of treatment 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

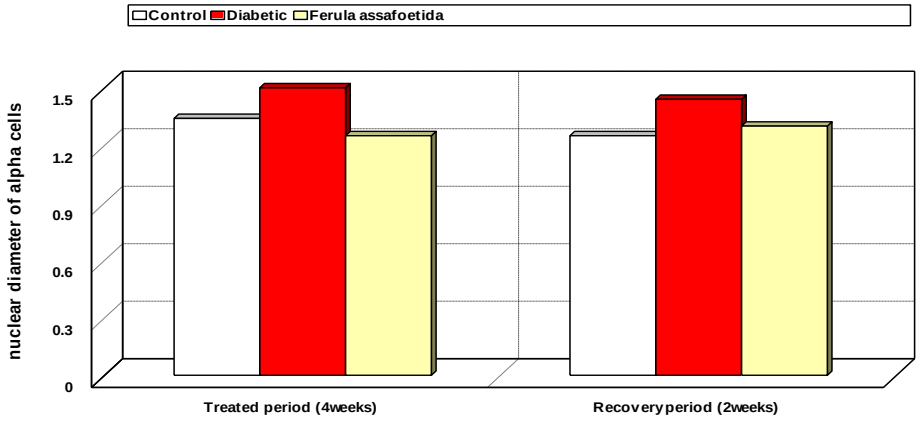


Fig.(10a): changes in the nuclear diameter of alpha cells in the islet of Langerhansin control, diabetic and plant extracts treated male albino rats after 4 weeks of treatment and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

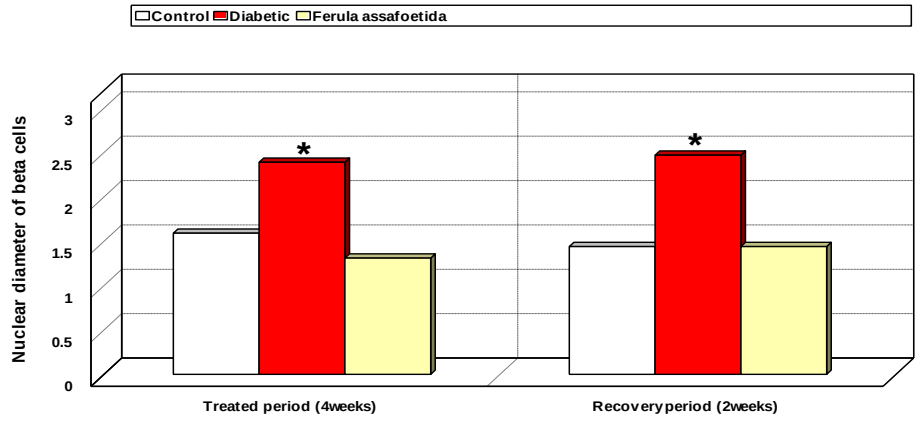


Fig.(10b):changes in the nuclear diameter of beta cells in the islet of Langerhanst in control, diabetic and plant extracts treated male albino rats after 4 weeks treatment and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

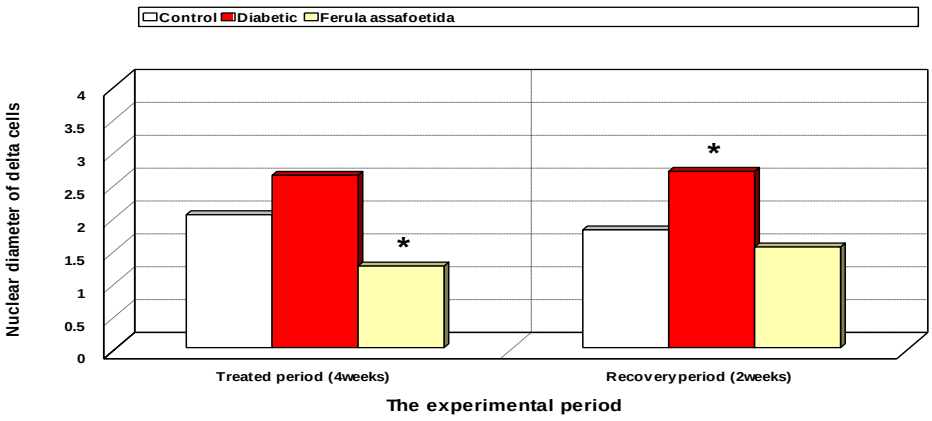


Fig.(10c): changes in the nuclear diameter of delta cells in the islet of Langerhans in control, diabetic and plant extract treated male albino rats after 4 weeks of treati and 2 weeks of recovery period.

(* = Significantat p< 0.05 - ** = Highly significant at p< 0.01)

Discussion

In the present study alloxan induction showed sever depression in body weight gain in comparison with normal rat, which may be due to depression of DNA and RNA synthesis in diabetic animals and/or different side effects of the ability to use carbohydrates including lypolysis and glycogenolysis and acidosis ***(Rawi et al., 1996, Abdel Moneim et al., 1999, Ganong, 2003 and Helal et al., 2005).*** And also recorded significant decrease in insulin secretion which may be due to the selective toxic effect of alloxan on the beta cells of islets of Langerhans **(*Bolaffi et al., 1986*).** Alloxan has direct inhibitory effect on ionic pump of the **β** cell membrane leading to increase in the cell size. It also inhibit intracellular energy production leading to decreased insulin synthesis and secretion ***(Majno and Joris, 1999)***.

The decreased liver glycogen in diabetic rats may be due to the increase of glycogenolysis with increased liver glucose output during insulin deficiency ***(Gold ,1970)***. It also may be due to decreased glycogenesis as a result of decreased glycogen synthetase activity and /or increased activity of glucose-6-phosphatase **(*Sheela and Augusti, 1992*)**.

The present study showed diminished size of islets of Langerhans and great damage in β cells in diabetic rats. This may be due to the deleterious effects of alloxan on permeability, transport, intracellular energy generation and insulin secretion which should be attributed to free radical formation which damage various cellular constituents and cytoplasmic vaculation ***(Malaisse, 1982)***. Vaculation of the cells is the most prominent lesion associated with functional islet abnormality and development of hyperglycemia ***(Bolaffi et al., 1986)***. And may be also attributed to the ability of alloxan to inhibit enzymes of the tricarboxylic acid cycle and Ca^{+2} dependent dehydrogenases in β cell mitochondria, causing ATP deficiency, cessation of insulin production and cell necrosis ***(Shafrir, 2003).***

Otherwise, the treatment of diabetic rats with Ferula assafoetida recorded an increase in the body weight gain when compared with control group. This increase in the body weight gain may be due to the activities of these plants in strengthening the gastrointestinal tract by increasing both the rate of secretion of the digestive juices and the motility of the gastrointestinal tract **(*Guyton,*** and ***Hall, 2000*)**, so they are taken for indigestion **(*Chevallier, 1996*** and ***Duke, 2002*)**. And also it caused a significant hypoglycemic effect which may be due to the Asafetida's volatile oil, which contains disulphids like that of garlic ***(Chevallier, 1996)***. Since their hypoglycemic action may be derived from disulphids which have been showed to be active in animals and humans. It has been investigated that, disulphids act as sparing agents for insulin by competing with it for inactivating compounds ***(Evans, 2001),*** or may be due to increase cellular utilization of glucose rather than acting on a pancreatic islets of Langerhans ***(Jain and Vyas,1975).***

The hypoglycemic activity of Ferula assafoetida may be also attributed to its content of sodium ferulate which is a potent antioxidant purified from Ferula assafoetida ***(Lu et al., 1998)***. Antioxidants in diabetics have been shown to improve whole body glucose levels and decrease fasting blood glucose in non-insulin dependent diabetes ***(Erixsson, 1995).*** The increase in liver glycogen content after treatment with plant extract is a result of increased leptin, amylin or adeponictein which decrease resistin has a potent effect on glycogen synthetase activity as well as on hepatic hexokinase and glycogen-6-phosphatase activity **(*Sheela*** and ***Augusti, 1992*)**.

It seems that supplementation of Ferula assafoetida immediately after the diagnosis of diabetes may delay the complications of diabetes. The preset study suggests that Ferula assafoetida is the one of choice, so it could not be used, a single medicine but it may be used in combination with other herbs in treatment of diabetes mellitus.

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التأثير الخافض للسكر لنبات الحلتيت على ذكور الجرذان البيضاء المصابة بالسكر التجريبي .

أيمان جمال الدين عزت هلال* ، أشرف محمود مصطفى أماني فوزي محمود فهمي *** أنوار الكامل كحوش ***

قسم علم الحيوان– كلية العلوم*– قسم الهيستولوجى** –كلية الطب– جامعة الأزهر

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فى هذا البحث أستخدم ثلاثون جرذا ابيضاً لدراسة تأثير الحلتيت على أيض الكربوهيدرات فى داء السكري ، قسمت إلي ثلاث مجموعات كل منها يحتوى علي عشر جرذان. المجموعة الأولى اعتبرت مجموعة ضابطة ، والمجموعة الثانية تم حقنها بالألوكسان لإحداث داء السكري ، والمجموعة الثالثة تم معالجتها بالحلتيت لمدة شهر بعد إحداث داء السكري بها . وقد تم قياس نسبة السكر وهرمون الأنسولين في الدم و الجليكوجين في الكبد لكل المجموعات. وكذلك متابعة وزن الجسم وعمل فحص مجهرى لشرائح البنكرياس. كما تضمنت الدراسة ايضا تقييم نفس القياسات بعد فترة الأستشفاء (خمسة عشرة يوما بدون أي علاج إضافي) .

وقد أدى الحقن بعقار الألوكسان الى تدمير خلايا بيتا مما أدى إلى نقص ذى دلالة إحصائية في هرمون الأنسولين و جليكوجين الكبد مع زيادة ذات دلالة إحصائية في نسبة السكر فى الدم.

وقد أ دت المعالجة بالحلتيت إلى تجديد خلايا بيتا مع زيادة في هرمون الأنسولين و جليكوجين الكبد وتحسن فى وزن الجرذان مع انخفاض في نسبة السكر فى الدم وقد امتدت هذه النتائج الإيجابية حتى بعد توقف العلاج – أى بعد فترة الاستشفاء مقارنة بالمجموعة المصابة بمرض السكري.

ويستخلص من هذا البحث أن لنبات الحلتيت تأثيراً جيداً على خلايا بيتا وافراز الأنسولين وتحسن وزن الجسم وجليكوجين الكبد وخفض نسبة السكر فى الدم ونوصى بمزيد من الأبحاث بجرعات مختلفة من النبات وكذلك دراسة الأثار الجانبية للنبات – إن وجدت– على أعضاء الجسم المختلفة قبل الوصول إلى رأى قاطع حول استخدام هذا النبات لعلاج مرض السكر.