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The Effects of *Androctonus Amoreuxi* Scorpion Extract and Sitagliptin in the Treatment of Diabetes Mellitus type 2 in Animal Models

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**ABSTRACT**

Diabetes mellitus (DM) is a common disorder associated with markedly increased morbidity and mortality rate. The purpose of the present study is to investigate the effect of daily administration of *Androctonus (A.) amoreuxi* scorpion extract and sitagliptin (Januvia) on blood glucose level, glycated hemoglobin and lipid profile as well as histopathological and immunohistochemical studies of pancreas in diabetic rats forty male albino rats (130-150 gm) were divided into four notequal groups: control, streptozotocin nicotinamide (STZ/NA) induced diabetic (DM) , diabetic rats treated with sitagliptin (DM+ST) and diabetic rats treated with scorpion extract (DM+Sc.Tea). Thirty days post ST and Sc. Tea administration (10 and 200 mg/kg B.Wt. respectively), biochemical and histological parameters were investigated. Diabetic rats exhibited significant increase in fasting blood sugar (FBS), glycated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), and a decrease in the level of high density lipoprotein (HDL). The administration of ST and Sc.Tea had shown significant decrease in FBS, HbA1c, TC and TG. However, a significant increase in HDL-cholesterol was recorded. Additionally, the beneficial effect of A. amoreuxi scorpion extract and sitagliptin was confirmed with histopathological examination of pancreas. Results of this study suggest that A. amoreuxi scorpion extract and sitagliptin have protective and anti-hyperglycemic effects.

**INTRODUCTION**

Diabetes mellitus (DM) is a common disorder associated with markedly increased morbidity and mortality rate. It can be defined as a group of metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both resulting in impaired function in carbohydrate, lipid and protein metabolism (Tierney, 2002; Zhang et al., 2006). Several pathogenic pathways are activated in diabetes among which reactive oxygen species (ROS)
generated by high glucose levels are responsible for metabolic abnormalities and chronic complications (Aronson, 2008).

In modern medication, the beneficial effects of drugs on glycemic levels are well documented but their preventive activity against progressive nature of diabetes and its micro and macrovascular complications are not always effective (Kasiviswanath et al., 2005). Moreover, synthetic antioxidants are suspected to be carcinogenic and hence are in no more use (Lavhale and Mishra, 2007). Therefore, search for appropriate antihyperglycemic agents has been used in traditional medicine because of leads provided by natural products that may be better treatment than currently used drugs (Hu et al., 2003).

Most therapeutic plans still focus on lowering blood glucose levels or the number of diabetic complications. Indeed, protection or regeneration of beta cells should be considered the key strategies to the treatment of DM. Scorpion in combination with other Chinese medications are mainly used to treat diabetic neuropathy (Zhang et al., 2008). Scorpions are also prescribed as an anticonvulsive, pain-relieving, anticoagulant, anticancer and immune regulating medication in Chinese traditional medicine (Luo et al., 2008). There have also been claims which indicated that scorpion combined with other Chinese medicines is effective in the treatment of type2 DM patients (Li et al., 2001). Based on previous studies pertaining to the therapeutical properties of scorpion venom, the following research was conducted to examine the effect of whole body extract of the scorpion Androctonus (A.) amoreuxi in the treatment of adult albino rats in which diabetes was induced by using streptozotocin nicotinamide (STZ/NA).

Januvia, glucose-lowering agent, is commonly used for the treatment of type 2 diabetes. Decreasing hepatic glucose production through gluconeogenesis suppression and activating peripheral glucose utilization in muscle, intestine and liver have been reported to be contributors to the glucose-lowering effect of Januvia (Sakr, 2013).

Therefore, the present study focused also on a comparison between the chronic effect of Januvia and A. amoreuxi whole body extract as a direct gluconeogenesis inhibitor and its subsequent effects on lipid profile, and glycated hemoglobin (HbA1c) of (STZ/NA -induced diabetic male rats. This could allow us to further understand the implications of the difference in mechanisms between Januvia and A. amoreuxi whole body extract in terms of clinical usage for the treatment of diabetes.

### MATERIALS AND METHODS

**Chemicals:**

Streptozotocin was purchased from Sigma-Aldrich (MO, USA), Nicotinamide (NA) was purchased from modern lab. Sitagliptin (Januvia® tablet) was obtained from Merck Sharp & Dohme Ltd (Pavia, Italy). Glimepiride was kindly provided from Medical Union Pharmaceuticals (Abu-Sultan, Ismailia, Egypt). All other chemicals and solvents were of highest analytical grade. The feed ingredients such as lard and sucrose were procured from the commercial sources. Citric acid, sodium citrate and sodium carboxymethyl cellulose (Na-CMC) were also obtained from ADWIC CO. (Cairo, Egypt).
Animals:
A total of 40 male albino rats Rattus rattus, were purchased from animal house of National Research Center (NRC), Doky, Cairo. These were specific pathogen free. Animals were treated according to ethical guidelines of NRC. The animals were housed in polystyrene cages (five animals per cage) throughout the study, and room temperature was maintained at 25 ± 2°C and at a 12-h light/12-h dark cycle. Food and water were allowed ad libitum. Animals were kept 2 weeks before starting the experiment for acclimatization.

Preparation of standard drug (Januvia)
Januvia; Generic Name: Sitagliptin Phosphate. Sitagliptin is a traditional anti-diabetic drug that works by increasing levels of natural substances called incretins. Incretins help to control blood sugar by increasing insulin release, especially after a meal. They also decrease the amount of sugar made by liver. Januvia (1000 mg/tablet) was purchased from a local pharmacy. Three tablets of drug were grinded to fine powder and dissolved and drugs were administered orally as a suspension in 1% sodium carboxymethyl cellulose (Na-CMC) solution and continued for a period of 30 days. Rat dose of Januvia was calculated from the standard clinical human dose on the basis of surface area \[ \text{rat dose} = \left( \frac{\text{human dose}}{\text{average body weight of rats}} \right) \times 7 \] (Freireich et al., 1966).

Preparations and whole body Androctonus Amoreuxi extract
About 100 A. amoreuxi scorpions were collected from North Western Egypt, around the Western Mediterranean Costal Desert in Alexandria. To lower the toxic effect, the toxic sting in the tail of the scorpion was discarded before its whole bodies were used for further preparations. First, the scorpions were dried overnight at 60°C and then reduced to a powder. The scorpion powder was soaked in warm water for 2 h and filtered through four sheets of gauze. The solid material was extracted twice again under the same conditions. The filtrates were combined and centrifuged to remove water-insoluble materials. After centrifugation for 15 min at 3000 r/min, the supernatant was lyophilized to yield the crude. The obtained crude was weighted and dissolved in saline solution and then became ready to use (Xie et al., 2010).

Induction of diabetes in rat
Diabetes was induced in 16 h fasted male rats by a single intraperitoneal injection of a buffered solution (0.1 M citrate, pH 4.5) of streptozotocin (STZ) and Nicotinamide (NA). Overnight fasted animals were administered NA (90 mg/kg) dissolved in 0.9% NaCl and after 15 min, STZ (60 mg/kg) dissolved in 0.1 mmol/l citrate buffer (pH 4.5) was injected. To prevent hypoglycemia, animals were given a 10% glucose solution for the next 48 h. Blood glucose level was measured 9 days after diabetes induction using reagent strips (Accu-Chek®, Roche). Blood was collected from tail vein and rats with blood glucose values more than 200 mg/dL were considered diabetics.

Experimental groups:
This work was carried out on 40 healthy male albino rats, with a body weight 130-150 gm. Animals were randomly divided into four groups, 10 rats in each group. Group I, normal negative control group 6 rats, intraperitoneally received isotonic saline (0.9% NaCl). Group II, STZ/NA-induced diabetic rats 21 rats. Group III, STZ/NA -induced diabetic rats and
intraperitoneally received standard drug treatment (Januvia) at a daily dose 10 mg/kg for one month. Group IV, STZ/NA-induced diabetic rats and intraperitoneally received treatment with scorpion extract at a daily dose of 200 mg/kg for 4 weeks according to Weidong et al. (2010). All animal groups were sacrificed one month post treatment, blood samples and pancreases were collected.

**Histopathology and immunohistochemistry**

Sections of 5μm thickness of pancreases that were fixed in 10% neutral buffered formalin were stained with haematoxylin and eosin and examined under light microscope according to Drury and Wallington (1980).

Immunohistochemical staining. Four-micrometer-thick paraffin sections were mounted on positively charged slides and subjected to the immunohistochemical (IHC) procedure using an Avidin-Biotin detection system (Ventana, Tucson, AZ, USA), following the manufacturer’s instructions using anti–glucagon and anti-insulin antibodies.

**Statistical analysis**

Statistics were calculated with SPSS for windows version 20.0, the means value obtained in the different groups were compared by one way ANOVA followed by Duncan's. All results were expressed as mean values ± SE and significance was defined as p< 0.05 (Field, 2000).

**RESULTS**

Normal levels of FBS and HbA1c in healthy adult rats were measured as 84.27±3.06 mg/dl and 2.99 ± 0.05, respectively. STZ/NA treated rats showed a significant elevation in FBS and HbA1c levels as compared with control group. Administration of Januvia (10 mg/kg b.wt.) and A. amoreuxi extract (Sc. Tea) (200 mg/kg b.wt.) caused significant reduction in blood glucose level (p≤ 0.05) as compared with diabetic control rats (Figs. 1 and 2 respectively). Also, they caused a significant reduction (p<0.05) in HbA1cas compared to diabetic rats.

![Fig. 1: Effect of Januvia or Androctonus amoreuxi extract (Sc. Tea) on blood glucose level in STZ induced diabetic rats. Values are means ± SEM. n=6, One Way ANOVA followed by Duncan multiple comparison tests. a p<0.05 compared with normal control group, b p<0.05 compared with Diabetic group.](image1)

![Fig. 2: Effect of Januvia or Androctonus amoreuxi extract (Sc. Tea) on blood Glycohemoglobin level in STZ/NA induced diabetic rats. Values are means ± SEM. n=6, One Way ANOVA followed by Duncan multiple comparison tests. a p<0.05 compared with normal control group, b p<0.05 compared with Diabetic group.](image2)
As shown in Fig. 3, there was a significant reduction (p ≤ 0.05) in insulin level in STZ/NA rats. However insulin level was significantly reversed by administration of Januvia and Sc. Tea administration when compared to the untreated diabetic group.

Table 1 showed the results of lipid profile of plasma in control and diabetic rats treated with Januvia and Sc. Tea. Data obtained revealed that lipid profile was impaired in STZ/NA -diabetic rats and the levels of TC and TG were significantly (p≤ 0.05) increased while the level of HDL was significantly (p ≤ 0.05) reduced compared with normal control group. Januvia treatment revealed significant decrease in total cholesterol (TC) and Triglyceride (TG), with percent of change -12.6 and -17.88 respectively compared with positive control (DM). However, High-Density Lipoprotein (HDL) showed significant increase with a percent of change 29.91 as compared with diabetic group. *Androctonus amoreuxi* extract treatment revealed significant decrease in total cholesterol (TC), Triglyceride (TG), with percent of change -16.7 and -14.63, However, High-Density Lipoprotein (HDL) showed significant increase with a percent of change 38.32 as compared with diabetic group.

Table 1: Effect of Januvia and *Androctonus amoreuxi* extract on lipid profile in STZ/NA induced diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.63± 2.72</td>
<td>16.70±1.42</td>
<td>46.80± 3.69</td>
</tr>
<tr>
<td>DM</td>
<td>56.00± 0.36</td>
<td>10.00 ± 3.01</td>
<td>65.00± 0.36</td>
</tr>
<tr>
<td>DM + Januvia</td>
<td>49.16± 10.42</td>
<td>13.87 ± 4.28</td>
<td>54.12 ± 13.90</td>
</tr>
<tr>
<td>DM + Scorpion extract</td>
<td>46.68 ± 6.50</td>
<td>14.00 ± 3.01</td>
<td>56.53 ± 5.40</td>
</tr>
</tbody>
</table>

Values are means ± SEM. n=6, One Way ANOVA followed by Duncan multiple comparison tests. 
*a p<0.05 compared with normal control group, *b p<0.05 compared with Diabetic group. *c p<0.05 compared Januvia group with *Androctonus amoreuxi* extract group.

The light microscopic examination of pancreas in control rats shows normal histological structure of the islets of Langerhans cells as endocrine and the acini as exocrine (Fig. 4A). The STZ/NA diabetic rats showed atrophy in the islets of Langerhans cells (Fig. 4B). However, treatment of STZ/NA -diabetic rats with either Januvia or Sc. Tea displayed recovery in size of islets of Langerhans (Figs. 4C and D, respectively).
Fig. 4: Photomicrographs of sections of the pancreas stained by H&E. A. Section from control group showing granulated cytoplasm of islet cells (IS) and acini (AC); B. Section from pancreas of a diabetic rat showing cytoplasmic degenerative changes in most islet cells, especially in center of the islet; C. Section from pancreas of group (STZ/NA + Januvia 10 mg/kg) presenting very similar morphology to the control group; D. Section from pancreas of a group (STZ/NA + scorpion extract 200 mg/kg) shows the nearly regular outline of an islet with apparently normal appearance of most cells. H&E staining, scalebar = 20 µm

Regarding the insulin expression in beta-cells (Fig. 5) and glucagon (Fig. 6) expression in alpha cells, STZ/NA-induced degenerative changes in islets led to decreases in the number of functioning beta and alpha-cells.

Fig. 5: Photomicrographs of Immunohistochemical localization of insulin expression in rat pancreases. A. Section of the pancreas of the control group showing strong immunoreactivity of insulin in beta-cells, which occupy most of the islet. B. Pancreas of a diabetic rat (STZ/NA) showing marked reduction in the immunohistochemical expression of insulin in beta-cells (arrow). C. In the pancreas of group (STZ/NA + Januvia 10 mg/kg), the apparent marked increase in number and area of beta-cells is evident in comparison with STZ/NA group. D. Section from rat of group (STZ/NA + Sc. Tea 200mg/kg) presenting an apparent increase in the number and area of beta-cells compared with STZ. scalebar = 20 µm.
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Fig. 6: Photomicrographs of Immunohistochemical localization of glucagon expression in rat pancreases. A. Section of the pancreas of the control group showing immunopositive cells in the islet of Langerhans of normal positive cells were observed in the peripheral region of the islets alpha cells were stained using Monoclonal Anti-Glucagon. B. Pancreas of a diabetic rat (STZ/NA) showing decrease and damage of of glucagon immunoreactivity and the number of immunoreactive α (arrow). C. In the pancreas of group (STZ/NA + Januvia 10 mg/kg), showing immunopositive cells in the islet of Langerhans of normal positive of number immuno positive cells in the islet of Langerhans of normal positive cells were observed in the peripheral region of the islets alpha cell with brown color glucagon α-cells increase in number. D. Section from rat of group (STZ/NA + Sc. Tea 200 mg/kg) presenting an apparent increase in the number and area of alpha-cells compared with STZ . scale bar 20 µm

In STZ/NA - treated rats, Januvia and scorpion Tea significantly decreased blood glucose concentration while increasing blood insulin levels. It also induced a significant increase in the number of immunoreactive beta- and alpha cells.

**DISCUSSION**

Diabetes mellitus is the most common endocrine disorder that affects more than 285 million people worldwide (Williams and Pickup, 2004). The number is expected to grow to 438 million by 2030, corresponding to 7.8% of the adult population. In addition to the primary effects of diabetes, this disease is accompanied by increased risk factors such as hyperglycemia, hypertension, dyslipidemia, decreased fibrinolytic activity, severe atherosclerosis and increased platelet aggregation (Rajalakshmi *et al*., 2009). Oral hypoglycemic agents currently used have serious side effect, hence there is a need to search a newer anti-diabetic agents that having high therapeutic efficacy with minimum side effect. This may be fulfilled by treating DM with traditional medicine anti diabetic agents (Holman and Turner, 1991).

The aim of the present study was to evaluate the anti-diabetic effects of the *A. amoreuxi* whole body extract in STZ/NA -induced type 2 diabetic rats. Streptozotocin- nicotinamide induced type 2 diabetes in rats provides a
relevant model to further elucidate the pathobiology of this disease. Animal models mimicking the pathology of human type 2 diabetes are of great value. One example of a type 2 diabetes animal model is STZ/NA -treated rat model (Skovsø, 2014).

In the present work, plasma glucose concentration was significantly elevated in STZ/NA induced-diabetic rats and this was associated with low plasma insulin level. This result could be due to the cytotoxic effect of STZ which cause a massive reduction of the β-cells of the islets of Langerhans in pancreas of STZ induced diabetic rat comparable to control. These observations were matching with the histological finding in the current work as diabetic mice pancreas were reduced in the islets size, decreased in the number of insulin secretory β cells and irregular shape accompanied with lymphatic infiltration of islets of Langerhans. Pancreatic β-cell death is fundamental in the pathogenesis of type 2 diabetes (Xing et al., 2009). On the other hand, decreased blood glucose content in the current study in scorpion extract treated diabetic mice and increased insulin level could be due to increase in the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway or regeneration of β-cells in pancreatic islets (Kumar et al., 2009). Thomson et al. (2007) reported that the hypoglycemic action could possibly be due to an increase in pancreatic secretion of insulin from β-cells and release of bound insulin or enhancement of insulin sensitivity. The majority of islet cells are formed by β-cells which are responsible for producing insulin. STZ is selectively destroying β-cells of the islets, so it was used to induce type2 diabetes (Xing et al., 2009). The mechanisms of pancreatic islet cell death in STZ model diabetes may be not only apoptosis but necrosis also could be involved (Skovsø, 2014).

Furthermore, Weidong et al. (2010) found that the treatment with scorpion and gypsum extract significantly increased the expressions of both peroxisome proliferator-activated receptor (PPARγ) in addition to pancreatic and duodenal homeobox-1 (PDX-1). PPARγ activation restores beta cell function in diabetic mice through the reduction of endoplasmic reticulum stress and maintenance of euchromatin structure (Evans et al., 2009). PPARγ has a regulating effect on PDX-1 transcription (Gupta et al., 2008). PDX-1 governs the early embryonic development of the pancreas and the later differentiation of insulin-producing islet beta cells of the endocrine compartment (Oliver et al., 2009). These results indicated that scorpion extract might exert a hypoglycemic effect by enhancing the PPARγ and PDX-1 expressions and promoting regeneration or proliferation of pancreatic islets β-cells.

Diabetes mellitus is associated with hyperlipidemia with profound alteration in the concentration and composition of lipids (Odetola et al., 2006). Data in the current work showed marked increase in plasma total cholesterol and triglycerides and in STZ-induced diabetic mice group in comparison to control group and this was in agreement with Okamoto et al. (2008). Scorpion extract treated diabetic mice group showed significantly decrease in plasma total cholesterol and triglycerides contents compared with diabetic group. Diabetic dyslipidaemia induced diabetic rats by elevated triglycerides, cholesterol, low density lipoprotein (LDL) and decreased HDL, constitutes an important cardiovascular risk factor (Okoli et. al., 2010). Both serum lipid and blood glucose concentrations were positively correlated (Miller and Wilson, 1984). This finding is supported by our finding that the increase in blood glucose concentration was accompanied by increasing in plasma cholesterol and triglycerides content by increasing in plasma cholesterol and triglycerides content due to defect in insulin secretion and/or action.
Lipid metabolism disorders following the glucose metabolism disorder is the direct cause of high hypertriglyceridemia and hypercholesterolemia, then they became the main factors that accelerate atherosclerosis and vascular complications in diabetes. Therefore, reducing the blood lipid level is a goal of delaying the complications of diabetes (Tang et al., 2006). STZ causes diabetes which leads to a massive reduction in insulin release by the destruction of β-cells of the islets of Langerhans, thereby, inducing hyperglycaemia and hyperlipidemia as consequence (Kusunoki et al., 2000). Many observations indicate that the hypo-cholesterolemic action of many extracts is attributed to the ability to suppress cholesterol biosynthesis. Furthermore, correlation between insulin levels, triglycerides and cholesterol fractions underline the important role of the hormone in the control of blood lipid levels. Indeed hepatic Very low density lipoprotein and triglyceride synthesis and secretion are regulated by insulin (Marles and Farns, 1995).

It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. Hence, STZ induced diabetic rats have altered lipid profile. In this study, diabetic control rats exhibited significantly elevated cholesterol and triglyceride as compared to normal control rats. Chronic administration of scorpion-extract and januvia significantly reduced all the previously mentioned parameters. Therefore, normalization of lipids in diabetic rats treated with scorpion-extract may be partly due to its stimulatory effect on insulin secretion from pancreatic β-cells (confirmed by serum insulin levels).

In STZ model, our histological findings showed significant decrease number of cells/islet, reduction in density and diameter of islets in pancreatic tissue observed in diabetic rats. The observations also show islet necrosis which is evidenced by heavy lymphocytic infiltration in and around the islet (Shirwaikar et al., 2006). Administration of the scorpion-extract and Januvia to the diabetic rats for 30-days caused enhancing the histological changes of islets of Langerhans and enhanced the expression of glucagon and insulin in pancreatic tissue. This may be due to the presence of some chemical compounds such as phospholipase A2 and its action can be related to possessing insulin-like action and had ability to induce DNA repair systems due to antioxidant activities which reduce or prevent generation of free radicals.

The present study confirms findings of Xie et al. (2005) in that A. amoreuxi scorpion venom could be used in treating diabetes. Also, the whole body extract of the scorpion used in the study had antidiabetic effect. Hence, it can be concluded that even whole body extract of the scorpion can produce therapeutic effects against hyperglycemia.

In conclusion, this study supported the hypothesis that hyperglycemia activated cellular and tissue damage and hyperlipidemia. Supplementation of natural remedies may be useful for treatment of diabetes. Scorpion extract (scorpion tea) exhibited antioxidant property could ameliorate the alternations induced in diabetes. The possible mechanisms by which scorpion extract brings about its hypoglycemic action may be due to (i) possible regeneration effect on pancreatic β-cells and induction of insulin secretion from β-cells of islets of langerhans and (ii) enhanced transport of blood glucose to peripheral tissues.

REFERENCES


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ARABIC SUMMERY

التأثير العلاجي المحتمل لمستخلص جسم العقرب أندركتونس أموريكسي وعقار سيتاجلبيتين في علاج مرض السكر - النوع الثاني في حيوانات التجربة

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داء السكري هو مرض شائع مرتبط بشكل ملحوظ بمعدلات الاعتدال ومعدل الوفيات. والمرض من هذه الدراسة هو فحص تأثير المعالجة اليومية بمستخلص جسم العقرب أندركتونس أموريكسي وعقار سيتاجلبيتين (جوفانيا) على مستوى السكر في الدم، الدهون، والهيموجلوبين السكري. وذكرنا دراسة الأنسجة والمناعية من البنكرياس في الجرذان المصابة بداء السكري. تم تقسيم الرباعين من ذكور الجرذان البيضاء (١٣٠-١٥٠ جم) إلى أربع مجموعات متساوية: المجموعة الضابطة، المجموعة المعالجة بالستربتوزوتين (STZ)، المجموعة المعالجة البداية بالسكري، والمجموعة المعالجة البداية بالسكري والعلاج بعقار سيتاجلبيتين (DM + ST).

النتائج أظهرت أن الجرذان المصابة بداء السكري، ومعالجة بعد يومين من البداية بالسكري، وعقار سيتاجلبيتين، ومستخلص جسم العقرب للمعالجة لمدة ثلاثين يوما. وجدت الدراسة أن الفئران المعالجة بعد يومين بالسكري، وعقار سيتاجلبيتين، ومستخلص جسم العقرب المستودوم بالسكري، وعقار سيتاجلبيتين، ومستخلص جسم العقرب المستودوم بالسكري، علامة الكثافة (HbA1c) والدهون الثلاثية (TG) ومستوى الهيموجلوبين السكري (HbA1c) الأكسدة مضادات الأكسدة وتخطيط التعبير الجيني للكولسترول والجلوكاجون في نسج البنكرياس استعداد توزيع الطبيعي بعد العلاج لمرض السكري المستثاث في الجرذان.