Effect of Apitoxin on Alveolar Bone Alterations in Induced Diabetic Rats (An Animal Study)

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a metabolic disorder caused by deficient insulin secretion or action or both. Among the main complications of DM is altered bone metabolism. Apitoxin, or honey bee venom (BV), has recently been suggested to have beneficial therapeutic effects against various diseases, including diabetes.

Objective: Evaluation of the possible therapeutic effect of BV in ameliorating diabetes-induced alterations in rat’s alveolar bone.

Materials and Methods: 18 adult male rats, about 3-4 months age, were utilized. 6 rats served as control (Group I), DM was induced in the remaining rats through streptozotocin injection. Diabetic rats were randomly divided into: Group II (treated with vehicle), Group III (treated with BV). All rats were sacrificed after 1 month, mandibles were examined histologically, by scanning electron microscopy and by RT-PCR to assess osteoprotegerin (OPG) and interleukin (IL)-17 genes’ expression.

Results: Histologically, group I showed a normal alveolar bone structure, while following diabetes induction, in group II, degenerative changes were noticed. Treatment with BV (group III) resulted in a relatively well restored histological structure of alveolar bone. Scanning electron microscopic results revealed a smooth buccal cortical plate in group I, while in group II, a generalized destruction was noticed. However, in group III, the bone surface demonstrated slight roughness. Statistical results presented a significant decrease in OPG gene expression in both group II and III than in group I. However; OPG gene expression was significantly increased in group III in comparison to group II. A significant increase occurred also in IL-17 gene expression in groups II and III. On the other hand, this expression significantly decreased in group III than in group II.

Conclusion: These results could suggest that BV treatment could ameliorate diabetes-induced alveolar bone changes; this could be attributed to the anti-osteoclastogenic and anti-inflammatory effects of BV.

Received: 22 June 2022, Accepted: 02 August 2022

Key Words: Apitoxin, bee venom, diabetic rats, IL-17, osteoprotegerin.

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ISSN: 1110-0559, Vol. 46, No. 4

INTRODUCTION

Diabetes mellitus (DM) is a diverse group of disorders characterized by elevated blood glucose levels. Type 1 DM is caused by an absolute insulin deficiency, mostly due to auto-immune pancreatic β cells’ destruction (1). By 2025, it is expected that about 300 million people worldwide will be suffering from diabetes, with a prevalence of about 6.4% (2). DM has conventionally been regarded as an adult metabolic disorder; yet, it has lately come to be more common among adolescents and, sometimes, children (3). Diabetic complications include: macro- and micro-vascular complications, retinopathy, neuropathy and nephropathy. In addition, bone metabolism is disturbed by diabetes, owing to diabetes-induced metabolic and endocrine changes which eventually affect quality and quantity of bone later during the patients’ life (4).

Various anti-diabetic drugs are currently used, but; unfortunately, they have many side effects including their cost. Thus, it is necessary to explore alternative anti-diabetic therapies from natural products like honey bee products, essentially apitoxin, also known as bee venom (BV). BV therapy is the medical usage of BV obtained from honeybees in the treatment of some human disorders (5). In alternate medicine, this approach has been anciently used for more than 5000 years, either indirectly through injecting extracted BV into the body or directly through bee stings (6). BV is a complex mixture of peptides, such as melittin, adolapin, apamin, mast cell degranulating peptide; amines including histamine, noradrenaline and dopamine; enzymes like hyaluronidase, phospholipase A2 as well as various carbohydrates and lipids (7).

BV proteins, essentially melittin, are generally used in the treatment of arthritis, central and peripheral nervous systems’ diseases, heart and blood related diseases, skin diseases, cancer, ulcer, and neuritis (8-11). Recently, in animal models, melittin was proven to significantly decrease blood glucose through insulin secretion and uptake of glucose (12). In addition, it exerts a significant lipid regulating action via phospholipase A2 activation. Thus, in the current study, we aimed to investigate the potential therapeutic effect of BV in minimizing DM-induced alterations in the rat’s alveolar...
bone. This was achieved through histological and scanning electron microscopic examinations, as well as assessment of osteoprotegerin (OPG) and interleukin (IL)-17 genes’ expression via quantitative RT-PCR.

MATERIALS AND METHODS

Animals

Eighteen male albino rats, with an average weight of about 150 to 200 gms and an average age of 3-4 months, were obtained from the animal house, Faculty of Medicine, Cairo University, and they were bred there. The animals were fed standardized laboratory diet and were given water ad libitum.

Experimental Design

Six rats served as control (Group I), while each of the remaining rats received a single intra-peritoneal (IP) injection of streptozotocin (STZ) freshly prepared in citrate buffer (pH 4.5) at a dose of 60mg/kg in order to induce diabetes.

Three days following STZ injection, rats with random blood glucose of >300 mg/dl were defined as diabetic[13]. Afterwards, diabetic rats were equally distributed as follows:

- **Group II**: Each diabetic rat was daily injected each with a single IP dose of 1 ml distilled water for 4 weeks.
- **Group III**: Each diabetic rat was daily injected each with a single IP dose of 0.5 mg/kg BV for 4 weeks[14].

BV Extraction

BV was collected from colonies of Apis mellifera honey bees (Italian and Carniolian hybrid), in the summer corn season in July, using a BV collector device at the National Research Center[15]. Dried BV was solubilized in distilled water to reach a concentration of about 0.1mg/ml to be injected into diabetic rats.

Investigations

At the endpoint of the experiment, rats from different groups were euthanized by anaesthetic overdose. Lower jaws were then dissected out; each was divided into two halves. Right halves were used for histological examination, while the left ones were used for both scanning electron microscopic (SEM) examination as well as molecular analysis through quantitative real time-PCR.

Light Microscopic Examination

Samples were fixed in 10% buffered formalin for 48 hours. Decalcification was then performed using 15% ethylene diamine tetra-acetic acid (EDTA) for 4 weeks. After decalcification was completed, samples were rinsed with buffer solution, dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sagittal sections of 4-6 um were cut, mounted on glass slides, stained with Haematoxylin and Eosin stain then examined under the light microscope[16].

Scanning Electron Microscopic Examination

Samples were fixed in 2.5 % glutaraldehyde in phosphate buffer (PH 7.3) and rinsed twice in the same buffer. Specimens were then dehydrated for 1 hour in a graded series of aqueous ethanol solutions containing 50%, 70%, 90%, and 100% ethanol. Then, the specimens were air-dried, then mounted on SEM stubs and finally they were examined using a scanning electron microscope (Quanta FEG 250/ EDS, Octane Pro, USA) to observe the alveolar bone surface integrity from the buccal aspect, using magnification X600[17].

Quantitative Real Time-PCR (qRT-PCR) Analysis

Total RNA isolation kit (Qiagen, USA) was used to isolate total RNA from the obtained samples, according to the manufacturer’s instructions. Following the protocol provided with the kit, RNA extracted from the samples was reverse-transcribed by cDNA Reverse Transcriptase kit (Fermentas, USA). The cDNA was then amplified and analyzed using the applied Biosystem with software version 3.1 (Step One™, USA). Using the comparative ΔΔCT method, relative mRNA gene expression was standardized according to the mean critical threshold values of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the housekeeping gene[18]. The list of primers for OPG, IL-17 and GAPDH genes was demonstrated in Table 1.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Primer sequence (From 5’ to 3’)</th>
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<tbody>
<tr>
<td>OPG</td>
<td>F: TGTGCGAATGCAAGGAAGAAGAAG</td>
</tr>
<tr>
<td></td>
<td>R: TGTATTTCGCTCTGGGGTTC</td>
</tr>
<tr>
<td>IL-17</td>
<td>F: CTTCACTGTGGCACTCT-GAGC</td>
</tr>
<tr>
<td></td>
<td>R: TGGCAGAACTAGGAGAAAC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: CGAGCGAAGGTCAACCGGC</td>
</tr>
<tr>
<td></td>
<td>R: GGTGTTGAGGACGC-CAGTA</td>
</tr>
</tbody>
</table>

Statistical Analysis

All obtained data from qRT-PCR were expressed as mean and standard deviation and were statistically analyzed using statistical package for Social Science (SPSS version 10). Comparison between studied groups were done using analysis of variance (ANOVA) followed by multiple pairwise comparisons using Tukey’s post hoc test. P-values less than 0.05 were considered as statistically significant.

Ethical Statement

The experiment was conducted at the animal house of the Faculty of Medicine, Cairo University, according to the permission of the Institutional Animal Care and Use Committee (IACUC) - Cairo University (CU III F 61 21).

RESULTS

Light Microscopic Results

- **Group I**: Histological examination of the alveolar bone of the control group revealed normal histological structure.
The interface between the alveolar bone proper and the periodontal ligament was relatively smooth, with no evident osteoclasts. The alveolar spongiosa demonstrated bone trabeculae enclosing multiple variable sized marrow cavities. The bone trabeculae contained regularly aligned osteocytes entrapped in their lacunae. The marrow cavities were occupied by vascular fibrocellular tissue and were lined by osteoblasts (Figure 1).

**Group II:** Regarding the diabetic group, the alveolar bone revealed various histological changes. Bone-periodontal ligament interface was noticeably irregular with lots of concave Howship’s lacunae. The spongiosa displayed bone trabeculae with obvious areas of degeneration as revealed by the presence of hyalinization, areas of empty lacunae, and even areas of complete absence of osteocytes in addition to the presence of focal irregular bony clefts. Intervening fatty marrow spaces were also seen, some of them were obviously widened and they showed discontinuity in their osteoblastic lining (Figure 2).

**Group III:** Examination of sections obtained from the diabetic group following treatment with BV showed that, the alveolar bone had a relatively well restored histological structure as compared to group II. An almost smooth bone-periodontal ligament interface was noticed. Regular bone trabeculae enclosing few small cellular marrow spaces were demonstrated. The bone trabeculae also revealed multiple resting lines. Quite well organized osteocytes were visible in their lacunae (Figure 3).

**Scanning Electron Microscopic Results**

**Group I:** The SEM images of the control group showed smooth, regular, uniform architecture of the buccal cortical plate of the alveolar bone. Well defined nutritive canal was also demonstrated (Figure 4a).

**Group II:** Following diabetes induction, a generalized destruction was noticed as demonstrated by the obvious roughening as well as irregular pits and grooves detected all over the surface of the buccal cortical plate. In addition, few collagen fibers were visible and some nutritive canals had widened and irregular outline (Figure 4b).

**Group III:** The alveolar bone surfaces of diabetic rats treated with BV demonstrated an almost restored architecture. They appeared with only slight roughness of the bone surface (Figure 4c).

**Statistical Results of Quantitative RT-PCR (Table 2)**

A significant difference in OPG mRNA gene expression between all studied groups has been revealed ($p<0.001$). A significant decrease in OPG gene expression was demonstrated in both the diabetic group (group II) and the BV treated diabetic group (group III) ($p<0.001$ & $p=0.007$ respectively) as compared to the control group (group I). However; OPG gene expression was significantly increased in group III in comparison to group II ($p<0.001$).

Regarding IL-17 mRNA gene expression; a significant difference was detected among all studied groups ($p<0.001$), where its expression was significantly higher in groups II and III as compared to group I ($p<0.001$). On the other hand, a significant decrease in IL-17 gene expression was noticed in group III than that in group II ($p<0.001$).
Fig. 2: Photomicrographs of the untreated diabetic group (group II). (A) Alveolar bone (AB) with irregularly arranged osteocytes, fatty marrow (M), irregular bone-periodontal ligament interface (arrows). (H&E, Orig. Mag.x200). (B) Degenerated bone trabeculae, with hyalinization and areas of complete absence of osteocytes (asterisks), trabecular cleft (c), Howship's lacuna (arrow). Inset: a higher magnification of Howship’s lacuna. (H&E, Orig. Mag. x400). (C) Trabecular hyalinization, with complete absence of osteocytes (asterisk), empty osteocytic lacunae (arrows), marrow cavities lack continuous osteoblastic lining (star). (H&E, Orig. Mag. x400).

Fig. 3: Photomicrographs of the BV treated diabetic group (group III). (A) Alveolar bone (AB), small marrow space (zigzag arrow), slightly undulated bone-periodontal ligament interface (arrows). (H&E, Orig. Mag.x200). (B) Relatively regular bone trabeculae (BT) with quite well organized osteocytes, resting lines (arrows), cellular marrow cavities (red arrows). (H&E, Orig. Mag. x400).

Fig. 4: Scanning electron micrographs of the buccal cortical plate of the alveolar bone. (SEM, Orig. Mag.x600). (A) Control group (group I): smooth bone surface (asterisk) with well-defined nutritive canal (arrow). (B) Untreated diabetic group (group II): rough eroded bone surface (asterisks), collagen fibers (arrows) and irregularly outlined nutritive canal (star). (C) BV treated diabetic group (group III): homogenous slightly roughened bone surface (asterisk).
**DISCUSSION**

DM is a serious metabolic disorder causing several organs dysfunction\(^{[19]}\). It has multiple long-term complications, the severity of which was generally dependent on the degree of hyperglycemia\(^{[20]}\). Despite the recently prevalent research and advanced treatments of DM; yet, it is still a major healthcare problem. Therefore, scientists have paid a great attention towards natural remedies, like BV, to be used as innovative therapeutic agents, which motivated us to conduct this study in order to explore the possible therapeutic effect of BV on alveolar bone of induced diabetic rats. BV has been traditionally used for treating various diseases\(^{[21]}\). It is made up of a complex mixture of various components, such as: polypeptides (melittin and apamin), enzymes (phospholipase A2 and hyaluronidase), and low molecular weight compounds (dopamine and histamine)\(^{[22]}\).

In the current study, obvious histological changes were demonstrated in the alveolar bone of diabetic rats when compared to the healthy control ones. This was reflected by trabecular deterioration with loss of normal bone structure, as well as osteocytic degeneration as evidenced by the presence of empty lacunae. These findings were coincident with previous researches that reported deleterious effects of diabetes on alveolar bone. These include significant bone loss and increased number of inflammatory cells\(^{[23,24]}\). Additionally, the histology of the bone marrow noticed in diabetic rats in the herein study was in accordance with previously reported diabetic bone marrow alterations such as microvascular rarefaction and fat deposition\(^{[25]}\). This may be owing to the enhancing effect of hyperglycemia on adipogenic differentiation of mesenchymal stem cells and fat deposition in the marrow cavity\(^{[26]}\).

Furthermore, in some areas of the alveolar bone, we demonstrated widened marrow cavities with discontinuous osteoblastic lining. This may be explained according to the previous findings of Gennaro et al.\(^{[26]}\) who reported that diabetes resulted in a reduced number of runt-related transcription factor 2-positive cells, which is a well-known osteoblastic differentiation regulator. Besides, in the present work, the existence of multiple Howship's lacunae along the bone-periodontal ligament interface in the diabetic group could indicate a significant osteoclastic activity, which is consistent with previous studies demonstrating increased osteoclastogenesis in hyperglycemic conditions\(^{[27,28]}\).

Interestingly, investigating the alveolar bone surface topography in the present study, using the scanning electron microscope, has greatly supported the obtained histological effects of diabetes on bone. This was revealed by the generalized destruction as shown by the obvious roughening and erosion detected all over the surface of the buccal cortical plate, as well as widening and irregular outline observed in some nutrient canals.

According to previous reports, diabetes-induced alveolar bone alterations could be attributed to increased osteoblasts apoptosis\(^{[29]}\), decreased expression of osteoblastic differentiation transcription factors as well as increased number of receptor activator of nuclear factor kappa-B ligand (RANKL)-positive cells\(^{[30]}\), eventually leading to imbalanced bone formation processes.

On the other hand, in the current study, following treatment of diabetic rats with BV, the alveolar bone histology was greatly restored. This could be evident through the presence of regular bone trabeculae having organized osteocytes in their lacunae. Moreover, the presence of smooth interface between the alveolar bone and the periodontal ligament suggested a reduced osteoclastic activity. An existent bone formation could also be assumed based on the presence of many resting lines, as well as presence of few small marrow cavities, which may infer that an excessive bone formation could have taken place on the expense of the marrow cavities. Inspecting the surface of bone in the specimens obtained from diabetic rats, following treatment with BV, revealed an obviously smooth bone surface with only minor surface roughness as compared to the diabetic rats.

From the current results, we could suggest an obvious potentiality of BV to prevent, or at least, to minimize the significant bone loss caused by diabetes induction. The beneficial effect of BV in reducing bone resorption has been demonstrated in various studies. For instance, Kwon et al.\(^{[30]}\) demonstrated that BV treatment significantly suppressed pathological bone damage in arthritic joints. Later, Choe and Kim\(^{[31]}\) reported that melittin, the major component of BV, hindered osteoclastic formation in RAW 264.7 cells and bone marrow-derived macrophages. The authors suggested that this inhibitory effect was mediated through inhibiting the RANKL-RANK system as well as attenuating the osteo-clastogenic influence of IL-1β. In addition, the work done by Gu et al.\(^{[32]}\) revealed that BV treatment reduced in vivo P. gingivalis-induced inflammatory alveolar bone loss-related periodontitis and it also significantly inhibited RANKL-induced differentiation, activation, and function of osteoclasts in vitro.

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**Table 2:** Mean ±SD of OPG and IL-17 mRNA genes’ expression in all studied groups

<table>
<thead>
<tr>
<th>mRNA gene expression</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPG</td>
<td>1.006±0.0089a</td>
<td>0.296±0.1009b</td>
<td>0.756±0.1521c</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>IL-17</td>
<td>1.002±0.0045a</td>
<td>4.136±0.4684b</td>
<td>2.874±0.2593c</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

*denotes a significant difference between all studied groups using ANOVA.

Means sharing different letters in the same row are statistically significant from each other as revealed by Post hoc Tukey’s test.
These findings also came in accordance with many former studies demonstrating the ability of BV, with its various components, to reduce blood glucose level and to diminish diabetic complications. These include the early work done by Morgan and Montague who demonstrated that melittin treatment resulted in reduced blood glucose levels through stimulating pancreatic β-cells to secrete insulin as well as helping the uptake of glucose. Besides, the authors attributed the main anti-diabetic role of BV to melittin, which also was proven to decrease diabetic complications via improving lipid profiles. Other anti-diabetic mechanisms of melittin include: β-cell membrane depolarization, as well as causing increased extracellular calcium, activation of cytosolic phospholipase A2, enhancement of uptake of glucose transporter lipid into adipose tissues and reduction of β-cell inflammation. Additionally, Kim et al. declared that BV was able to prevent DM in non-obese diabetic mice owing to its immune-modulating actions. They also confirmed that treatment with BV didn’t cause any complications throughout their whole investigation, which could point out to its primary safety.

OPG, a glycoprotein produced by osteoblasts and marrow stromal cells, is a potent inhibitor of osteoclast cells. It could inhibit the differentiation and maturation of osteoclasts and promote apoptosis of osteoclasts, which is achieved through its competitive binding to receptor activator of RANKL, a primary mediator of differentiation of osteoclasts. OPG mRNA gene expression was investigated in the present study as a possible indicator of the degree of inhibition of bone resorption. Our results revealed a significantly decreased OPG expression in the alveolar bone of induced diabetic animals (group II) suggesting an enhanced activation of osteoclasts resulting in a greater activation of bone resorption. These findings are consistent with those of Gennaro et al. who evaluated the immunohistochemical expression of OPG in induced diabetic rats and they demonstrated a statistically significant reduction in the number of OPG positively stained cells in diabetic rats. More recently, Qi et al. reported that in diabetic rats, OPG expression levels were lower, RANKL expression levels were higher, and the OPG/RANKL ratio was lower, indicating that bone resorption was stronger than bone formation in case of diabetes.

On the other hand, treating diabetic rats with BV in our study (group III) has resulted in a statistically significant up-regulation of OPG gene expression as compared to the diabetic rats (group II). This finding suggests that BV could possess a significant anabolic effect on bone, as this up-regulation was coupled with a histologically detected attenuation of diabetes-induced bone loss. In consistency with our results, the number of OPG positively stained cells in alveolar bone were significantly increased following the treatment of diabetic rats with green tea than the case in the untreated diabetic ones. The enhanced expression of OPG could attribute to hindering bone loss via direct binding to RANKL; so, blocking RANK receptor signaling in preosteoclasts. As a result of these regulatory systems, osteoclast multiplication and survival, as well as bone resorption, would be reduced.

IL-17 is a pro-inflammatory cytokine produced by T-helper 17 lymphocytes. Dis-regulation of IL-17 could result in excessive pro-inflammatory cytokine production as well as chronic inflammation; eventually causing tissue damage and autoimmune response. In the current work, IL-17 gene expression in the induced diabetic rats (group II) was significantly increased, compared to the control group. This was in agreement with former reports demonstrating that diabetes was associated with elevated levels of IL-17. A positive relationship between IL-17 and bone loss had been demonstrated, where IL-17 was reported to induce bone loss via increasing pro-osteoclastogenic cytokines from osteoblasts, including TNF-α and RANKL, as well as promoting osteoclastogenesis through upregulating RANKL expression by osteoblasts. Additionally, IL-17 activates NF-κB in osteoblasts, which, in turn, can inhibit the differentiated function of these cells.

Going through our molecular results, the increased gene expression of IL-17 associated with diabetes (group II) was significantly attenuated by BV treatment (group III). These results suggest an anti-inflammatory effect of BV. In general, several studies have verified the anti-inflammatory action of BV, for example, in a collagen-induced arthritis rat model; BV was proposed to hinder the production of cytokines like IFN-γ, IL-1β and TNF-α in addition to delaying disease progression. Furthermore, it was reported to suppress edema in addition to IL-6 levels. Recently, a study performed on an induced Parkinson’s disease mouse model revealed that in phospholipase A2 treated mice; the motor function was improved than that in the untreated controls. In addition, phospholipase A2 treatment inhibited the damage of dopaminergic neurons in these animals. The authors proposed that these actions were attributed to stimulating regulatory T cell differentiation, together with inhibiting the differentiation of inflammatory T-helper 1 and T-helper 17 cells. These findings could explain why, in the current investigation, IL-17 gene expression was significantly reduced following treatment of diabetic rats with BV, which hence, may be attributed to the inhibitory effect of BV on T-helper 17 cells.

Conclusively, from the obtained results in the herein study, it could be deduced that treatment of diabetic rats with BV, has resulted in an obvious improvement of the histological and scanning electron microscopic features of alveolar bone. The positive effect of BV treatment might be attributed to its anti-osteoclastogenic and anti-inflammatory effects. Hence, an obvious potentiality of BV to ameliorate diabetic induced alveolar bone loss could be strongly suggested. However, safety of its use is still questionable, since it is usually injected subcutaneously, which could result in adverse reactions such as swelling, edema and local erythema. Thus, further in vivo animal studies are necessary to evaluate the long term safety of BV.
BV before validating its usage as an efficient adjuvant for anti-diabetic therapy in any clinical trials.

**CONFLICT OF INTERESTS**

There are no conflicts of interest.

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الملخص العربي

تأثير الأبيتوكسين على تعديلات العظام السنخية في الجرذان المصابة بداء السكري
(دراسة حيوانية)

إيمن أبو شادي 1، دينا فرج 1

الجامعة الحديثة للتكنولوجيا والمعلومات، مصر

مرض السكري هو إضطراب التمثيل الغذائي الناجم عن نقص إفراز أو عمل الأنسولين أو كليهما. من ضمن المضاعفات الرئيسية لمرض السكري تغير التمثيل الغذائي للعظام. تم مؤخرًا اقتراح أن الأبيتوكسين، نحل العسل، له أثر علاجية مفيد ضد الأمراض المختلفة، بما في ذلك مرض السكري.

الهدف من التجربة: تقييم التأثير العلاجي المحتمل لسم النحل في تخفيف التغيرات التي يسببها مرض السكري في العظم المحيط بأسنان الجرذان.

المستخد: 18 جرذان ذكور بالغين. استخدمت 6 فئران كمجموعة تحكم (المجموعة الأولى) ، تم التسبب في مرض السكري في الفئران المتبقية من خلال حقن الستربتوزوتوسين. تم تقسيم الفئران المصابة بمرض السكري بشكل عشوائي إلى: المجموعة الثانية )تركت دون علاج( ، المجموعة الثالثة )معالجة بسم النحل(. تم التضحية بجميع الفئران بعد شهر واحد ، وتم فحص الفك السفلي من الناحية الهستولوجية ، عن طريق المسح المجهري الإلكتروني.

النتائج: من الناحية الهستولوجية، أظهرت المجموعة الأولى بنية عظام طبيعية محيطة بالأسنان ، بينما بعد التسبب بمرض السكري ، لوحظت تغيرات منكسة في المجموعة الثانية. نتج عن العلاج بسم النحل (المجموعة الثالثة) بنية نسيجية جيدة نسبيًا للعظم المحيط بالأسنان. كشفت نتائج المسح المجهري عن صفائح قشرية شدقي ناععة في المجموعة الأولى ، بينما لوحظ تدمير عام في المجموعة الثانية. لاحظت الخلايا في المجموعة الثالثة، أظهر سطح العظم خشونته طفيفة. قدمت النتائج الإحصائية ارتفاعًا كبيرًا في التعبير الجيني للأوستروبروتيجين في كل من المجموعتين الثانية والثالثة مقارنة بالمجموعة الأولى. تم زيادة التعبير الجيني لأوستروبروتيجين بشكل ملحوظ في المجموعة الثالثة، لكن هذه التغييرات كانت أقل في المجموعة الثانية وسابقة. من ناحية أخرى، انخفضتدرجه من الناحية الهستولوجية، في المجموعة الثالثة.

الخلاصة: يمكن أن تشير هذه النتائج إلى أن العلاج بسم النحل يمكن أن يحسن تغذية العظام المحيطة بالأسنان التي يسببها مرض السكري.