

Hypoglycemic effect of an extract from date seeds on diabetic rats

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ABSTRACT

الأهداف: للتحقق من كفاءة مستخرج مائي من أنوية التمر في علاج الجرذان البيضاء المصابة بداء السكري.

الطريقة: أجريت الدراسة بقسم التشريح، كلية الطب، جامعة الملك سعود خلال الفترة من 1 نوفمبر 2008م حتى 31 ديسمبر 2009م. وقد استخدم في هذه الدراسة 80 جرذ أبيض بالغ والتي تم توزيعها على 4 مجموعات. استخدمت جرذان المجموعة الأولى كمجموعة ضابطة. وأعطيت جرذان المجموعة الثانية جرعة يومية من المستخرج قدرها 10 ملليلتر بالفم. أصيبت جرذان المجموعتين الثالثة، والرابعة بداء السكري بواسطة حقنها بعقار ستربتوزوتوسين. ثم تم خضوع هاتين المجموعتين للعلاج بالأنسولين بجرعة قدرها 3 وحدات عالمية في اليوم لمدة 8 أسابيع. بالإضافة إلى إعطاء جرذان المجموعة الرابعة نفس الجرعة اليومية من المستخرج المعطاة للمجموعة الثانية. وقد تم قياس مستوى الجلوكوز الصائم بالدم لكل جرذ بمعدل مرة واحدة أسبوعياً كما أنه في نهاية التجربة تم قياس مستوى الهيموجلوبين (HbA1c) لكل جرذ.

النتائج: ولقد أوضحت النتائج وجود تغير مهم بين متوسط مستويات الجلوكوز بالدم للمجموعتين الثالثة والرابعة بدءاً من الأسبوع الثاني حتى نهاية التجربة. كما أوضحت النتائج أن مقارنة متوسط مستويات الجلوكوز بالدم لجرذان المجموعة الرابعة كل أسبوعين قد أظهرت انخفاضاً مهم حتى الأسبوع السادس، وأثبتت النتائج أن متوسط نسبة الهيموجلوبين لجرذان المجموعة الرابعة قد انخفض انخفاضاً ملحوظاً مقارنة بجرذان المجموعة الثالثة.

خاتمة: توضح هذه النتائج أن مستخرج أنوية التمر يعد مخفضاً لنسبة الجلوكوز بالدم، وأن هذا المستخرج مصحوباً بالأنسولين يؤدي إلى انخفاض نسبة جلوكوز الدم بدرجة ملحوظة في اتجاه النسب الطبيعية مقارنة مما ينتج عن استخدام الأنسولين بمفرده لعلاج الجرذان البيضاء المصابة بداء السكري.

Objectives: To investigate the efficacy of an aqueous extract from date seeds on diabetic rats.

Methods: The study was performed in the Department of Anatomy, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia between November 2008 and December 2009. Eighty adult albino rats were divided into 4 groups. Group 1 was used as healthy control. Group 2 was given daily ingestions of 10 ml of the date seed extract. Animals of groups 3 and 4 were made diabetic by injection of streptozotocin. Diabetic rats of group 3 received daily subcutaneous injections of 3 IU/day of insulin for 8 weeks while group 4 received ingestions of 10 ml of extract in addition to insulin. Fasting blood glucose levels were measured once weekly. Glycosylated hemoglobin (HbA1c) was also estimated.

Results: There is a significant change in the mean blood glucose levels between group 3 and group 4 from week 2. The mean blood glucose levels of group 4, every 2 consecutive weeks, showed a significant decrease until week 6. The HbA1c was significantly lower in group 4 compared to group 3.

Conclusion: The hypoglycemic effect of date seed extract combined with insulin, decreases the blood glucose level significantly toward normal when compared to the effect of insulin administered as a single drug for treatment of diabetes.

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Diabetes is a predominant public health concern affecting a large population in the whole world. The disease causes substantial morbidity, mortality, and long-term complications.¹ With increasing rates of childhood and adult obesity, diabetes is likely to become even more prevalent over the coming decade.² There is an increasing use of complementary and alternative medicine among the general public.³ In general, the scientific literature on the efficacy of alternative medicine in the treatment of diabetes is relatively sparse and heterogeneous. Studies have examined mind-body techniques,⁴ yoga, massage therapy,⁵ and other systems of healing such as traditional Chinese medicine.³ Most of the literature, however, has focused on testing the efficacy of certain herbs and other dietary supplements such as vitamins and minerals in the treatment of diabetes.⁶⁻⁸ Herbal remedies and other dietary supplements taken by mouth are considered among the top alternative therapies used, regardless of the sample surveyed.⁹ Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world.¹ However, there is insufficient evidence to actively recommend the use or ensure the safety of any particular supplement.² Palm dates are the major fruit crops in the Kingdom of Saudi Arabia with an annual production of approximately 830,000 tons.¹⁰ Since the seeds constitute approximately 10% of the fruit,¹⁰ the utilization of the seeds as by-product of date industry in medicine would be of great interest. Date seeds have been used successfully in the folk medicine to treat diabetes mellitus for many years without scientific basis. The efficacy of the date seeds has not been tested before. The present study was designed to investigate the efficacy of an aqueous extract from date seeds in the glycemic control of diabetes mellitus in rats.

Methods. The study was performed in the Department of Anatomy, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia between November 2008 to December 2009. Eighty adult Sprague-Dawley albino rats weighing 250-300 gm were used in this study. The Deanship of Scientific Research, King Saud University, Riyadh, Kingdom of Saudi Arabia approved this research. Principles of laboratory animal care were followed, as well as specific national laws were applied. The study was first designed in 5 groups. Rats in the fifth group were diabetic taking daily ingestions of 10 ml of seed extract. However, most of animals of this group died in the first 2 weeks of experiment. Thus, we excluded all animals of group 5 from the study. Therefore, the remaining animals were divided into 4 groups of 20 each. Group 1 - was used as a control group that did not receive any treatment. Group

2 - was given a daily ingestion of 10 ml of the date seed extract. Group 3 - was treated with a daily subcutaneous injection of insulin. Group 4 - was treated with a daily subcutaneous injection of insulin in addition to a daily ingestion of 10 ml of the date seed extract. Diabetes mellitus was induced in groups 3 and 4. Each animal of these 2 groups received a single intravenous injection of freshly prepared streptozotocin (Sigma Chemical Co, St. Louis, Missouri, USA) dissolved in 0.1 mol/L citrate buffer (pH 4.5) at a dose of 60 mg/kg body weight.¹¹ Rats in groups 1 and 2 received an equivalent dose of the buffer. Animals with a blood glucose level higher than 300 mg/dl, 3 days after streptozotocin injection, were used in the experiment.¹² Diabetic rats in groups 3 and 4 were treated with a daily subcutaneous injection of insulin glargine (Lantus, 100 IU/ml; Sanofi Aventis, Frankfurt, Germany). The dose of insulin was 3 IU/day/rat, in 2 divided doses for 8 weeks. The dose of insulin was adjusted to maintain the life of animals (not to return blood glucose levels to normal). Blood samples were collected from overnight fasted rats at the beginning of experiment then at a weekly interval, and we also measured the blood glucose levels. At the end of experiment, blood glucose levels were measured using Ascensia contour blood glucose monitoring system (Bayer Health Care, Berkshire, UK). Glycosylated hemoglobin (HbA1C) content was determined for each rat, using kits obtained from the FlexSite Diagnostics, Palm City, Florida, USA.

Preparation of the date seed extract. Seeds obtained from "Sukkary" dates were washed with tap water, left to dry, roasted and crushed. Crushed seed powder was added to distilled water to make a mixture of 50g/L. The mixture was boiled until it becomes brownish in color then finally filtered.

Statistical Analysis. Results were expressed as mean \pm standard deviation (SD). The significance of the difference between the values from different groups was determined using the one way analysis of variance (ANOVA) combined with post-Hoc Dunnett's T3 test for multiple comparisons (when comparing mean blood glucose levels) and post-Hoc Bonferroni test (when comparing mean HbA1c levels). Paired-samples t-test was used to compare between mean blood glucose levels of Group 4 (seed extract + insulin-treated diabetic rats) at every 2 consecutive weeks. $P < 0.05$ was being defined as statistically significant. The statistical software for data analysis used was Statistical Package for Social Sciences version 16.0 (SPSS Inc, Chicago, Illinois, USA).

Results. Mean fasting blood glucose levels were significantly higher in insulin-treated diabetic rats (group 3) as compared to healthy controls (group 1) and seed extract-treated rats (group 2) throughout the

whole experiment. Similarly, mean blood glucose levels were significantly higher in seed extract + insulin-treated diabetic rats (group 4) as compared to groups 1 and 2 throughout the whole experiment. Comparing group 3 to group 4, no significant change could be detected between the 2 groups in the mean blood glucose levels at the beginning of experiment and at week one. However, the change became significant between the 2 groups beginning from week 2 onward. Comparing group 1 to group 2, no significant change could be detected between the 2 groups in the mean blood glucose levels throughout the whole experiment. Comparison between mean blood glucose levels of rats of group 4 at every 2 consecutive weeks showed a significant decrease until week 6. However, no significant change was detected when comparing blood glucose levels at week 6 to those at week 7, and when comparing levels at week 7 to those at week 8 (Table 1 & Figure 1).

The mean HbA1c level was significantly higher in rats of groups 3 and 4 when comparing each of them to those in groups 1 and 2. The mean HbA1c was significantly lower in group 4 when compared to that

in group 3. While no significant difference in the mean HbA1c levels was found between rats of groups 1 and 2 (Figure 2).

Discussion. Conventional hypoglycemic drugs are widely used.¹³ Considering insulin as an example of such drugs, its disadvantages were discussed before.¹⁴⁻¹⁶ The present study tried to get solid evidence on the efficacy of an aqueous extract from date seeds in the treatment of diabetes mellitus in rats. Compared to insulin, date seed extract is easily administered (by oral route), easily available, and almost costless. The present study showed that the mean blood glucose levels were significantly higher in groups 3 and 4 when compared to groups 1 and 2. Comparing group 3 to group 4, the latter showed a significant decrease in blood glucose levels beginning from week 2 onward. This indicates that seed extract has a hypoglycemic effect when combined with insulin. Also, there is a lag period of approximately 2 weeks between the time of administration of the extract and the manifestation of its effect. Perhaps this lag period with no apparent hypoglycemic effect was the possible

Table 1 - Mean blood glucose levels (mg/dl) \pm SD throughout the experiment.

Weeks	Group I	Group II	Group III	Group IV
0	106.7 \pm 9.1	105 \pm 5.2	437.3 \pm 25.7*	433.4 \pm 27.8*
1	105.4 \pm 7.7	104.6 \pm 6.6	343 \pm 28.4*	324 \pm 16.7*
2	107.8 \pm 5.4	102.3 \pm 5.4	319.3 \pm 17.3*†	263.2 \pm 11.3*
3	105.4 \pm 7.1	102.8 \pm 9.2	281.6 \pm 27.9*†	187.6 \pm 9.7*
4	106.2 \pm 8.1	101.5 \pm 8.3	293 \pm 18.5*†	174.8 \pm 6.6*
5	105.7 \pm 3.3	100.8 \pm 8.7	284.3 \pm 21.1*†	163.2 \pm 5.8*
6	107.6 \pm 6.2	99.4 \pm 11.3	287.7 \pm 19.5*†	140.6 \pm 5.4*
7	106.4 \pm 7.3	100.3 \pm 12.5	285.6 \pm 25.2*†	138.8 \pm 3.2*
8	107.3 \pm 6.4	99.7 \pm 13.5	286.5 \pm 13.2*†	138.1 \pm 2.3*

*significant versus groups I, II; †significant versus group IV

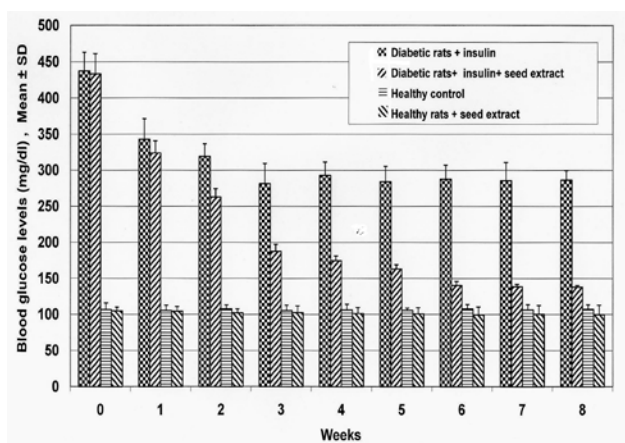


Figure 1 - Mean blood glucose levels (\pm standard deviation [SD]) of different groups.

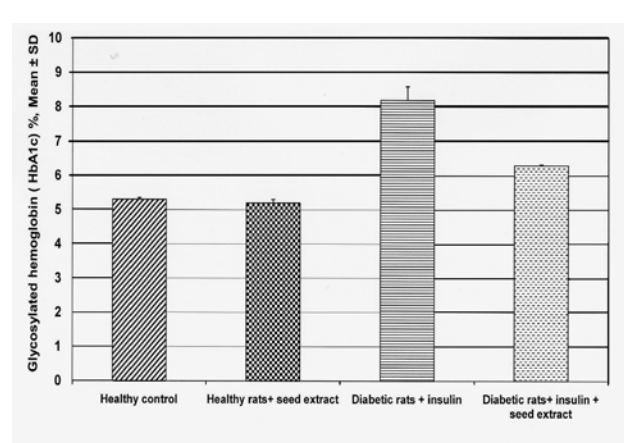


Figure 2 - Mean \pm standard deviation (SD) of glycosylated hemoglobin (HbA1c)% of different groups at the end of 8 weeks.

cause of high mortality rate of rats in group 5 (seed extract-treated diabetic rats). Animals of such group suffered from high blood glucose levels for a relatively long period without effective treatment. Comparison between mean blood glucose levels of group 4 at every 2 consecutive weeks showed a significant decrease until week 6, in which no significant change was detected. It might be possible that, for a given dose of seed extract, there is a peak for its hypoglycemic effect. When reached, blood glucose remains stable with no further decrease. Estimation of HbA1c is considered more reliable index of glycemic control in the management of diabetes mellitus than fasting blood glucose levels.¹⁷ The life span of a red blood cell in the albino rat is approximately 7-8 weeks.¹⁸ Therefore, measuring HbA1c can tell how high blood glucose has been on average over last 8 weeks. For this reason, we decided to terminate the experiment after 8 weeks. The mean HbA1c was found to be significantly lower in group 4 when compared to group 3. No significant difference was found between groups 1 and 2 in the mean HbA1c levels. This might indicate that the seed extract, when administered to healthy animals, did not show a significant hypoglycemic effect. However, when it was administered in combination with insulin to diabetic rats, blood glucose levels decreased significantly, compared to the group who used insulin as a single drug. In other words, one can say that insulin, when combined with date seed extract, might be used for better glycemic control, which might lead to the decrease of the dose of insulin given to diabetics. Regarding the potential mechanism of the date seed extract, it could be suggested that such extract might stimulate undifferentiated cells in pancreatic islets to differentiate into newly-formed β cells. The period taken by cells to differentiate and to secrete insulin might correspond to the lag period between administration of the extract and obtaining a response. Regarding the limitations of the study, the authors did not test the effect of multiple dosages of the extract on blood glucose levels in the different groups of the study and were just interested to investigate its efficacy. The study also lacks a histopathological investigation on pancreatic islets, as well as, a serological estimation of levels of endogenous insulin in all groups which could be beneficiary for suggesting the mechanism of action of the extract.

In conclusion, date seed extract has a hypoglycemic effect. Combined with insulin, blood glucose level decreases significantly toward normal when compared to effect of insulin administered as a single drug for treatment of diabetes. The response to date seed extract needs a lag period in order to manifest. A peak is achieved after which blood glucose level remains stable.

The present results would suggest further studies to investigate the effect of dosage variations of the extract on blood glucose levels of treated rats, and to determine the mechanism of action of the extract by studying the histopathological changes in β -cells of pancreatic islets and estimating endogenous insulin levels in treated rats. Also, investigation of possible side effects of the extract by studying the histopathological and biochemical changes in the vital organs such as liver and kidney of treated rats would be recommended. Such studies would be a preliminary step for testing its efficacy on human.

Finally, the results would also encourage the use of complementary and alternative medical therapy in Saudi Arabia for the treatment of diabetes mellitus.

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