An extract from date seeds having a hypoglycemic effect

Is it safe to use?

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ABSTRACT

الأهداف: التحقق من فعالية مستخلص نوى التمر ومقارنة التأثير السمي لداء السكري على الجرذان المعالجة بالأنسولين فقط والجرذان المعالجة بالأنسولين مع مستخلص نوى التمر بالمقابل.

الطريقة: أجريت الدراسة في قسم التشريح، كلية الطب، جامعة الملك سعود، الرياض، المملكة العربية السعودية خلال الفترة من أغسطس إلى ديسمبر 2010م. وقد تم تقسيم 100 جرذ إلى 5 مجموعات، بحيث تمثل المجموعة الأولى المجموعة الضابطة، كما أعطيت جرذان المجموعة الثانية جرعة يومية من مستخلص نوى التمر قدرها 10 ملليلتر بالفم. تم إصابة جرذان المجموعتين الثالثة والرابعة بداء السكري بحقنها بمادة ستربتوزوتوسين، كما تم خضوع هاتين المجموعتين للعلاج بالأنسولين بجرعة مقدارها 3 وحدات عالمية يومياً لمدة 8 أسابيع بالإضافة إلى إعطاء جرذان المجموعة الرابعة مقدارها 10 ملليلتر من مستخلص نوى التمر (نفس الجرعة اليومية من مستخلص نوى التمر المعطاه للمجموعة الثانية). كما تم إصابة جرذان المجموعة الخامسة بداء السكري بواسطة حقنها بمادة ستربتوزوتوسين وإعطائها الجرعة اليومية من المستخلص فقط. وفي نهاية التجربة، تم قتل الجرذان وفصل المصل من كل منها لقياس مستوى أنزيمات الكبد (ألانين أمينوترانسفراز، وأسبرتات أمينوترانسفراز، وجاما جلوتميل ترانسفراز)، ومعدل نيتروجين البولينا، والكرياتين بالدم. كما تم إعداد الكبد والكلى للفحص بواسطة المجهر الضوئي.

النتائج: أظهرت النتائج زيادة ملحوظة في متوسط مستويات أنزيمات الكبد ونيتروجين البولينا والكرياتين بالدم للمجموعة الثالثة مقارنة بالمجموعات الأولى والثانية والرابعة (فيما عدا أنزيم الألنين أمينوترانسفراز في المجموعة الرابعة)، بينما لم يكن هناك تغيراً ملحوظاً عند مقارنة المجموعات الأولى والثانية والرابعة بعضها البعض. ولقد أظهر الفحص المجهري للكبد و الكلى بالمجموعة الثالثة تغيراً خلوياً مرضياً، أما بقية المجموعات، فقد أظهر الفحص المجهري للكبد و الكلى عدم وجود تغيرات مرضية بها.

خاتمة: أوضحت النتائج آمان استخدام مستخلص نوى التمر على الكبد والكلى، كما يقلل هذا المستخلص مع الأنسولين من أضرار مرض السكري على هذين العضوين الهامين.

Objectives: To investigate the safety of date seed extract administration, and to compare between toxic effects of diabetes on rats treated with insulin versus rats treated with insulin-seed extract.

Methods: This study was performed in the Anatomy Department, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia from August to December 2010. One hundred rats were divided into 5 groups. Group 1 served as control. Group 2 was given daily ingestions of 10 ml of date seed extract. Animals of groups 3 and 4 were made diabetic by streptozotocin injection, and were given daily subcutaneous injections of 3 IU/day of insulin for 8 weeks. Group 4 received, in addition, daily ingestions of 10 ml of seed extract. Group 5 were made diabetic with streptozotocin and then given the seed extract only. At the end of experiment, rats were decapitated, and the sera were separated for estimation of alanine aminotransferase (ALT), aspartate aminotransferase, gamma glutamyl transferase, blood urea nitrogen, and serum creatinine levels. Livers and kidneys were processed for light microscopic study.

Results: The mean values of all tested serum levels were significantly higher in Group 3 compared to Groups 1, 2 and 4 (with the exception of ALT in the case of Group 4). There was no significant change when comparing the mean values of Groups 1, 2, and 4. Livers and kidneys of rats in Groups 1, 2, and 4 showed normal histology, while those of Group 3 showed histopathological changes.

Conclusion: Date seed extract administration is safe on the liver and kidney. In addition, insulin-date seed extract combination minimizes the toxic effects of diabetes on these organs.

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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] Conventional hypoglycemic drugs are widely used.[2] Considering insulin as the drug of choice for type 1 diabetes, its disadvantages were discussed in previous studies.[3,4] There is an increasing use of complementary and alternative medicine among the general public.[5] Date seeds have been used in folk medicine to treat diabetes mellitus (DM) for many years without scientific basis. The efficacy of an aqueous extract from date seeds has been tested successfully in the glycemic control of type 1 DM in rats.[6] Compared to insulin, date seed extract is easily administered (by oral route), easily available, and almost costless. The use of any medication might be associated with possible unwanted effects (side effects) on vital organs. Liver and kidney are important organs of metabolism, detoxification, storage and excretion, and therefore, are especially vulnerable to damage.[7-9] This work is carried out to provide information on the effect, if any, of the administration of date seed extract on the structure and functions of rat liver and kidney. It would also provide a comparison between the effect of diabetes on such organs, in cases of rats treated with a combination of insulin and date seed extract, and those treated with insulin as a single drug for the treatment of diabetes. The present study would be a preliminary step for testing the efficacy of such extract in the treatment of DM in humans.

Methods. This study was performed in the Department of Anatomy, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA) from August to December 2010. One hundred male adult Sprague Dawley albino rats weighing 250-300 gm were used in this study. The Deanship of Scientific Research, King Saud University, Riyadh, KSA approved this research. This study followed the International Guidelines for the Care and Use of Laboratory Animals. This study was first designed in 5 groups of 20 rats each. Rats in the fifth group were made diabetic by taking a daily ingestion of 10 ml of seed extract. However, most animals of this group died in the first 2 weeks of experiment. Thus, we excluded all animals of group 5 from the study. The rest of the animals (80 rats) were divided into 4 groups of 20 rats each, such as: Group 1 - control group that did not receive any treatment; Group 2 - was given a daily ingestion of 10 ml of the date seed extract for 8 weeks;[6] Group 3 - was treated with a daily subcutaneous injection of insulin; Group 4 - was treated with a daily subcutaneous injection of insulin, immediately followed by a daily ingestion of 10 ml of the date seed extract. Diabetes mellitus was induced in animals of Groups 3 and 4 by a single intravenous injection of freshly prepared streptozotocin (Sigma Chemical Co, St. Louis, Missouri, USA) at a dose of 60 mg/kg body weight, dissolved in 0.1 mol/L citrate buffer (pH 4.5).[10] Rats in Groups 1 and 2 received an equivalent dose of the buffer. Only rats with a blood glucose level higher than 300 mg/dl, 3 days after streptozotocin injection were used in the experiment.[11] Diabetic rats in Groups 3 and 4 were treated with a daily subcutaneous injection of 100 IU/ml insulin glargine (Lantus [Sanofi Aventis, Frankfurt, Germany]). The dose of insulin was 3 IU/day/rat in 2 divided doses for 8 weeks, and it was adjusted to maintain the life of animals (that is, not to return blood glucose to normal).[6] At the end of the experiment, blood glucose levels were measured using Ascensia contour blood glucose monitoring system (Bayer Health Care, Berkshire, UK).

Serum analysis. The animals were anesthetized with ether, and then decapitated. The blood of each rat was collected into a marked conical test tube, allowed to clot, and left for 10 minutes at room temperature for serum formation. The tubes were centrifuged at 3750 x g for 5 minutes. The sera were collected in appropriately labeled tubes, kept frozen, and used for various liver and kidney function analyses. Serum measurement of selected biochemical parameters was performed using Automated Biochemical Analyzer (Siemens Aktiengesellschaft, Munich, Germany). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (γ-GT) levels were estimated to test liver functions. Similarly, blood urea nitrogen (BUN) and serum creatinine levels were estimated to test kidney functions.

Histological study. The liver and kidneys of each animal were dissected out. The specimens were fixed in 10% formol saline then processed for paraffin sectioning. Sections of 5 µm thick were cut and stained with Hematoxylin and Eosin. All experimental work, chemical analysis, and histological interpretation were run under blind conditions.

Preparation of the date seed extract. Seeds obtained from "Sukkary" dates were washed with tap water, left to dry, roasted, and crushed. The crushed seed powder

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was added to distilled water to prepare a mixture of 50 g/L. The mixture was boiled until it becomes brownish in color, and then finally filtered.

Statistical analysis. Results were expressed as mean ± standard deviation (SD). The significance of the difference between the values from different groups was determined using the one way analysis of variance (ANOVA) followed by the post-Hoc Duncan test for multiple comparisons. Results were considered significant when $p \leq 0.05$. Data were analyzed using the Statistical Package for Social Sciences version 16.0 (SPSS Inc, Chicago, Illinois, USA).

Results. ***Biochemical results.*** **Table 1** shows the results of liver and kidney function tests, expressed as mean ± SD in the 4 groups.

Liver function tests. The mean values of serum AST and serum γ-GT were significantly higher in rats of Group 3 (insulin-treated) when compared to Groups 1, 2, and 4. No significant change was detected in the mean values of serum AST and serum γ-GT when comparing Groups 1, 2, and 4 with each other. The mean value of serum ALT was significantly higher in Group 3 compared to Groups 1 and 2, but not statistically significant when compared to Group 4. No statistically significant differences were observed when comparing the mean value of serum ALT of Groups 1, 2, and 4 with each other.

Kidney function tests. The mean values of both BUN and serum creatinine were significantly higher in Group 3 compared to Groups 1, 2, and 4. No statistically significant differences were observed when comparing Groups 1, 2, and 4 with each other.

Histological results. Liver. Stained liver sections from rats of Groups 1, 2, and 4 showed normal hepatic architecture. Hepatocytes were arranged in

Table 1 - Results of liver and kidney function tests (mean ± standard deviation) in the studied groups.

Groups	ALT (U/L)	AST (U/L)	γ-GT (U/L)	BUN (mg/dl)	Creatinine (mg/dl)
1	45.575 ± 5.875	52.050 ± 6.283	65.350 ± 2.831	17.125 ± 3.037	0.4250 ± 0.079
2	45.775 ± 7.561	51.925 ± 5.239	66.550 ± 3.623	17.600 ± 3.102	0.4275 ± 0.045
3	54.300 ± 2.815*	69.050 ± 6.140†	78.475 ±6.409†	28.225 ± 3.551†	1.3400 ± 0.414†
4	50.450 ± 2.812	53.275 ± 4.496	67.025 ± 2.394	19.975 ± 1.063	0.6650 ± 0.113

*significant compared to Groups 1 and 2, †significant compared to groups 1, 2 and 4 ($p<0.05$). ALT - alanine aminotransferase, AST - aspartate aminotransferase, γ-GT - gamma glutamyl transferase, BUN - blood urea nitrogen

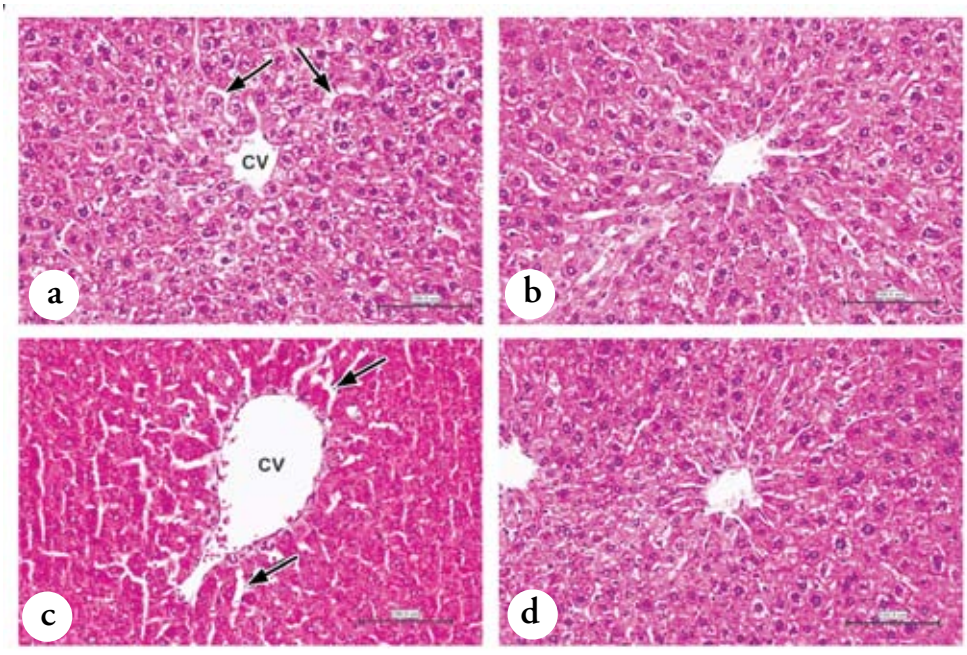

Figure 1 - Histological results showing: **a**) section of the liver from the control rat (Group 1), showing normal hepatic architecture with normal central vein (CV), and plates of hepatocytes separated by normal hepatic blood sinusoids (arrows). **b**) Section of the liver from the group receiving seed extract only (Group 2), showing normal hepatic architecture. **c**) Section of the liver from a diabetic rat injected with insulin (Group 3), showing some dilatation of the CV and blood sinusoids (arrows). **d**) Section of the liver from a diabetic rat injected with insulin and received seed extract (Group 4), showing normal hepatic architecture. Hematoxylin and eosin stain, scale bars 100 µm.

plates radiating from the central veins. These plates of hepatocytes were separated by blood sinusoids (**Figures 1a, b, d**). Liver sections from Group 3 showed dilatation and congestion of both the central veins and the blood sinusoids (**Figure 1c**).

Renal cortex. Stained sections of the renal cortex from rats of Groups 1, 2, and 4 showed normal renal corpuscles, and proximal and distal renal tubules. The renal corpuscles showed patent capsular space with no proliferation of mesangial cells. Sections of the proximal

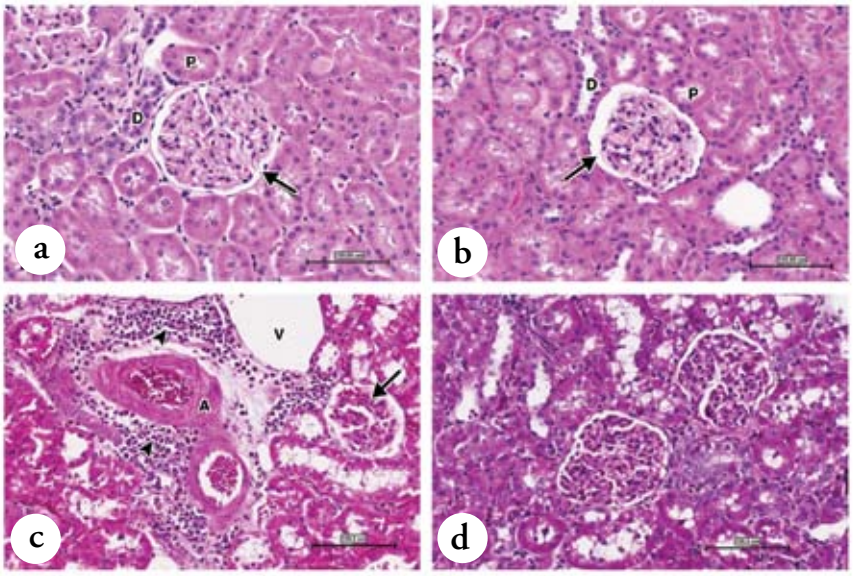

Figure 2 - Histological results showing: **a**) section of the kidney cortex from a controlled rat (Group I), showing a normal renal corpuscle with patent capsular space (arrow) and numerous proximal (P) but fewer distal (D) convoluted tubules, **b**) section of the kidney cortex from a control rat receiving seed extract only (Group 2), showing normal renal cortex architecture, arrow - capsular space; P - proximal convoluted tubules, D - distal convoluted tubules, **c**) section of the kidney cortex from a diabetic rat injected with insulin (Group 3), showing thickening of the arterial walls (A), venous dilatation (V), perivascular mononuclear cellular infiltration (arrowheads), and shrinkage of some glomeruli (arrow). **d**) section of the kidney cortex from a diabetic rat injected with insulin and received seed extract (Group 4), showing almost normal renal cortex architecture (Hematoxylin and Eosin stain, scale bars 100 μm).

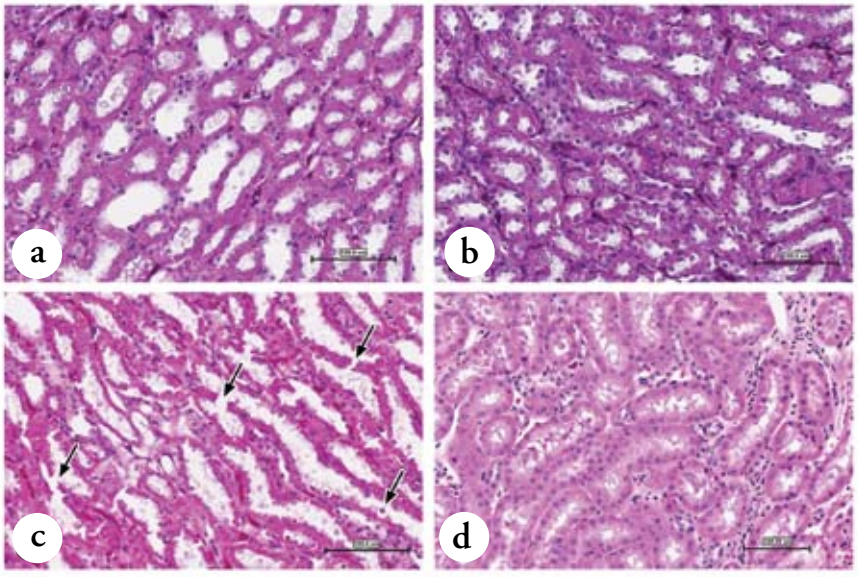

Figure 3 - Histological results showing: **a**) section of kidney medulla from a control rat (Group 1), showing normal collecting tubules, **b**) section of kidney medulla from a control rat receiving seed extract only (Group 2), showing normal collecting tubules, **c**) section of kidney medulla from a diabetic rat injected with insulin (Group 3), showing disintegration of tubular cells with their necrosis and desquamation (arrows), **d**) Section of kidney medulla from a diabetic rat injected with insulin and received seed extract (Group 4), showing normal collecting tubules (Hematoxylin and Eosin stain, scale bars 100 μm).

convoluted tubules were more numerous in comparison to fewer sections of distal convoluted tubules (**Figures 2a, b, & d**). Sections of the kidney cortex from rats of Group 3 showed thickening of the arterial walls, venous dilatation, and perivascular mononuclear cellular infiltration. Many renal corpuscles showed shrinkage or segmentation of their glomeruli (**Figure 2c**).

Renal medulla. Stained sections of the renal medulla from rats of Groups 1, 2, and 4 showed normal renal collecting tubules with normal interstitium (**Figures 3a, b, d**), while sections from rats of group 3 showed marked degeneration, necrosis, and desquamation of collecting tubular epithelium (**Figure 3c**).

Discussion. The efficacy of an aqueous extract from date seeds has been tested successfully in the glycemic control of type 1 DM in rats.[6] The previous results encouraged the authors to conduct a study aiming to investigate the safety of the date seed extract administration by studying the biochemical and histopathological changes in the liver and kidney of healthy rats treated with the date seed extract. This study also aimed to compare between the effect of diabetes on liver and kidney of rats treated with the combination of insulin and date seed extract versus those treated with insulin as a single drug for treatment of diabetes.

Biochemical studies were performed to test the liver and kidney functions of rats in all groups. The biochemical indices evaluated in the present study are useful parameters to indicate impairment in the functional capacities of such organs. Liver functions were evaluated by measuring serum ALT, AST, and γ-GT activities. The levels of BUN and serum creatinine were estimated to assess the functional capacity of the kidney. The present results showed an increase in the mean values of the biochemical indices in the sera of rats of Group 3. Similar changes in the mean values of those indices in the case of diabetic rats[10] and humans[12,13] were reported before. The mean values of all parameters tested were significantly higher in rats of Group 3 compared to those of Groups 1 and 2. With the exception of serum ALT, a significant difference was also observed between the values in rats of Group 3, and those in rats of Group 4. No significant change was detected in the mean values of all parameters tested when comparing Groups 1, 2, and 4 with each other. Accordingly, there is no apparent toxic effect of the date seed extract on the liver and kidney functions of healthy rats when administered daily for 8 weeks. Furthermore, the use of a combination of insulin and date seed extract decreases significantly the mean values of all tested parameters toward normal, when compared with insulin used as a single drug, thus improving liver and kidney functions, and minimizing toxic effects of diabetes on such organs.

Histological results showed that the liver of healthy rats treated with date seed extract for 8 weeks (Group 2) exhibited normal hepatic architecture. The picture was more or less similar in the case of diabetic rats treated with insulin and date seed extract (Group 4). On the other hand, liver sections from diabetic rats treated with insulin (Group 3) showed histopathological changes in the form of dilatation and congestion of both central veins and blood sinusoids. Regarding the kidney, sections obtained from rats of Groups 2 and 4 showed normal renal cortex and medulla. However, sections of kidney from rats of Group 3 showed the picture of vasculitis with thickening of the arterial walls and perivascular mononuclear cellular infiltration. In addition, focal shrinkage of glomeruli, as well as, focal degeneration and necrosis of collecting tubular epithelium were also observed. Similar histopathological changes in liver and kidney of diabetic rats[14-16] and human[13,17,18] were reported before. The present results reported the absence of histopathological changes in liver and kidney of healthy rats taking a daily ingestion of date seed extract for 8 weeks (Group 2) indicating the safety of extract ingestion for the given period. Furthermore, the apparently normal histological picture of liver and kidney of rats of Group 4 versus the damage observed in rats of Group 3 would suggest that the combination of insulin and date seed extract minimize the histopathological effects of diabetes on liver and kidney when compared to insulin administration for the same period.

The effect of multiple dosages of the extract, as well as the effect of extract for longer durations was not tested, and this limits our study. Furthermore, histopathological data observed were not evaluated quantitatively. In addition, this study had aimed to include a fifth group of diabetic rats taking daily ingestion of 10 ml of seed extract alone, however, most animals died in the beginning of the experiment. A previous study[6] on the efficacy of date seed extract as a hypoglycemic agent reported a lag period of approximately 2 weeks between the time of administration of the extract and the manifestation of its effect, and suggested that this lag period with no apparent hypoglycemic effect was the possible cause of high mortality rate of rats in Group 5. Animals of such group might suffer from high blood glucose levels for a relatively long period without effective treatment.

In conclusion, date seed extract administration is apparently safe. Compared with insulin administration as a single drug, insulin-date seed extract combination minimizes the toxic effects of diabetes on the liver and kidney. The present results would encourage further studies to investigate the effect of dosage variations

and duration of administration of the extract on the structure and function of vital organs. Investigation of the mechanism of action, by which insulin-date seed extract combination minimizes the toxic effects of diabetes would be also essential to determine whether this is due to the additive hypoglycemic effect of the extract, and/or to an additional property of the extract.

The results would encourage testing of the efficacy of the date seed extract, as a supplement for insulin in the treatment of DM in humans.

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Related topics

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