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Ameliorative role of palm date seeds on pancreatic function in diabetic rats

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Abstract :

Background: Diabetes is a metabolic disease that affects the ability of pancreatic beta cells to produce insulin. **objective** This study aimed to investigate the vital role of palm date seed extract in improving pancreatic function in alloxan-induced diabetes rats. **Materials and Methods:** Twenty-four rats were housed in an animal facility for 30 days. The Rats were randomly distributed into four groups. The control group was administered distilled water, while the other three groups were administered alloxan to induce diabetes. The Third and fourth groups were treated with date seeds extract and metformin, respectively. The Results showed a significant increase in glucose, insulin and resistin levels along with a decrease in adiponectin levels in non- treated diabetic rats compared to control group. **Results:** Treated diabetic rats demonstrated increase insulin and adiponectin levels along with a decrease in glucose and resistin levels. Metformin showed no significant effect on resistin level compared to the non-treated diabetic group. Alloxan exhibited a destructive effect on pancreatic tissue such as a rupture of Langerhans islets and necrosis while the seeds extract reduced cells damage in serous glands. **Conclusion:** We concluded the vital activity of date seeds components and the pivotal role in reducing glucose levels and ameliorative insulin sensitivity and improving beta cell's structure and function in diabetic rats.

Keywords: palm date seed, Diabetes Mellitus, alloxan, pancreas, insulin sensitivity.

الدور التحسيني لمستخلص بذور التمر في وظيفة البنكرياس لدى الجرذان المصابة بداء السكري

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مستخلص:

الخلفية: يُعد داء السكري أحد الاضطرابات الأيضية المزمنة التي تؤثر في كفاءة خلايا بيتا في البنكرياس وقدرتها على إفراز الإنسولين. **هدف الدراسة**: تهدف هذه الدراسة إلى تقصي الدور الحيوي لمستخلص بذور التمر في تحسين وظيفة البنكرياس لدى الجرذان المصابة بداء السكري المستحث بإبادة الألوكسان. **طرائق العمل**: استُخدم في هذه الدراسة (24) جرذًا مختبريًا، وُضعت في ظروف قياسية داخل بيت الحيوان لمدة (30) يومًا، وقُسّمت عشوائيًا إلى أربع مجموعات. مثّلت المجموعة الأولى مجموعة السيطرة (Control) وأُعطيت الماء المقطر، في حين حُفِّز داء السكري في المجموعات الثلاث الأخرى باستخدام الألوكسان. عُولجت المجموعة الثالثة بمستخلص بذور التمر، بينما عُولجت المجموعة الرابعة بعقار الميتفورمين. **النتائج**: أظهرت النتائج ارتفاعًا معنويًا ($P\leq0.05$) في مستويات كل من الكلوكوز، والإنسولين، والريزستين، يقابله انخفاض معنوي في مستوى الأديبونكتين لدى الجرذان المصابة غير المعالجة مقارنةً بمجموعة السيطرة. في المقابل، بينت الجرذان المعالجة بمستخلص بذور التمر تحسنًا ملحوظًا تمثل بارتفاع مستويات الإنسولين والأديبونكتين، وانخفاض مستويات الكلوكوز والريزستين. كما لم يُظهر الميتفورمين تأثيرًا معنويًا في مستوى الريزستين مقارنةً بالمجموعة المصابة غير المعالجة. وأظهرت الدراسة النسجية أن الألوكسان سبب أضرارًا واضحة في نسيج البنكرياس، تمثلت بتهتك جزر لانغرهانس وحدوث تنخر، في حين أسهم مستخلص بذور التمر في تقليل الضرر الخلوي وتحسين البنية النسيجية. **الاستنتاج**: تشير نتائج الدراسة إلى أن لمستخلص بذور التمر فعالية حيوية في خفض مستويات الغلوكوز في الدم، وتحسين حساسية الإنسولين، فضلًا عن تعزيز سلامة التركيب النسيجي ووظيفة خلايا بيتا البنكرياسية لدى الجرذان المصابة بداء السكري.

الكلمات المفتاحية: بذور التمر، داء السكري، الألوكسان، البنكرياس، حساسية الإنسولين.

Introduction

Diabetes is a metabolic disease that affects the ability of pancreatic beta cells to produce insulin (1). Insulin deficiency causes hyperglycemia by preventing glucose from entering cells (2). The pancreas loses its ability to produce insulin via destruction of beta cells (3). Diabetes directly attenuates endocrine function of the pancreas due to insulin resistance or insulin deficiency, and indirectly affects the exocrine function of pancreas affecting overall health (4). Alloxan is a chemical compound induces diabetes through its ability to destroy beta cell (5). Alloxan used in scientific researches to study the pathophysiology and test antidiabetic drugs (6).

A date palm belonging to the Arecaceae family is a traditional medicine for fever, inflammation, neurological disorders, and dementia (7). The palm date seeds had a hard oval shape inside the dates. Recently, date seeds have gained attention owing to their nutritional value and potential health benefits. The antihyperglycemic, antioxidant, anti-inflammatory, hepatoprotective, and nephroprotective properties of date palm may be attributed to the presence of a wide range of phytochemicals, including oleic acid, linoleic acid, catechin, epicatechin, anthocyanins, ellagic acid, gallic acid, p-coumaric acid, coumarins, quercetin, rutin, myricetin, apigenin, naringenin, and chlorogenic acid (8).The therapeutic interventions to manage the disease include antihyperglycemic drugs such as metformin, sulfonylureas, and sodium-glucose co-transporter 2 (SGLT-2) inhibitors, which enhance insulin secretion and reduce insulin resistance, but are often accompanied by many side effects (9).

Metformin reduces blood glucose concentration by inhibiting hepatic glucose production, enhancing insulin sensitivity, and reducing fat storage in liver cells by activating AMP-activated protein kinase (AMPK) (10). Metformin is an oral hypoglycemic drug with a significant role in improving IR and lipid profile in diabetic persons without causing hypoglycemia.(11)

This study aimed to detect the ameliorative role of palm date seeds on pancreatic function in rats exposed to

induced diabetes and compare the results with metformin.

Materials And Methods

seeds collection and preparation

seeds were collected from date palms (Barhi) in the Al-Alam District at Salah al-Din Governorate/ Iraq. The samples were then washed , dried at room temperature, and ground to obtain a powder. the date seed powder was extracted using a cold aqueous method .(12).Experimental dose determined according to a pretest measured the ability of different concentrations of seed extract to reduce serum glucose level in rats.

Chemical analysis of Phoenix dactylifera seeds

Kildahl method was used to estimate the protein (13). fats were estimated based on the method of (AOAC 1995). The total carbohydrate content estimated using the method of (14). The elements were also estimated according to the method described by (15).

Induction of D.M by alloxan

Animals were starved for 18 hours before injected with alloxan 150 mg/kg. Alloxan was dissolved in normal saline and injected as a single dose sub peritoneal (16). Immediately after injection, animals were provided with food and glucose solution (10%) for 24 hours to prevent the shock of hypoglycemia (17). Glucose levels were examined every two days for ten days to confirm the occurrence of the injury (18).

Experimental design

Twenty-four of Sprague Dawley rats aged 10-12 weeks and weighed 190-210 g housed in animal unit facility in college of veterinary medicine at Tikrit university/Iraq. Rats exposed to standard laboratory conditions due period of experimental study. Eighteen of thirty rats infected with diabetes induced by alloxan 150 g/kg. Rats distributed into 3 groups firstly is healthy control CTRL administrated by distill water, secondly diabetic group D.M., thirdly diabetic rats treated by solution of date seeds 50% is the third group and fourthly is diabetic rats administrated by metformin.

At the end of the 30-days, blood samples were drawn by jugular vein. The blood was placed in test tubes and placed in a water bath at 37 °C for 30

minutes, then a centrifuge was used at 3000 rpm for 15 minutes to obtain serum.

Biochemical tests determination

Sera used for determine biochemical tests included levels of Glucose, Insulin, Resistin and Adiponectin. Tests made utilizing Enzyme Linked Immunosorbent Assay technique using kits supplied by Sunlog company. Principle of determination and calculation of concentrations set according to kit instructions.

Histological study preparation:

Pancreatic tissue directly fixed in 10% formalin for 24 hours, then washed using tap water followed by dehydration using a series of alcohol concentrations. The samples cleared, infiltrated, then embedded in paraffin. The paraffin-embedded tissue was sectioned to a thickness of 5µm using an electric rotary microtome. sections were stained using hematoxylin and eosin then examined under a light microscope (19).

Ethical approval

The study was conducted according to the ethical principles that have their origin in the Declaration of Helsinki. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee and registered under No. 2025.1.

Data analysis

Data customized in Graph pad prism 10.2.3 results expressed by means ± SD. Results analyzed by One-way ANOVA and the significant differences among means were estimated using Duncan multiple tests.

Results

- Biochemical results

Palm date seeds extract have a nutritional value. The chemical analysis of seeds appeared the seeds contain carbohydrates, proteins and lipids in addition to minerals as shown in table 1.

The study showed a significant increase in blood glucose, insulin and resistin levels and a significant decrease in adiponectin levels in the non-treated diabetic group compared to the control group. The groups treated with date palm seed extract showed an improvement in glucose, insulin, resistin and adiponectin levels compared to the non-treated diabetic group. Also,

treatment with metformin showed an improvement in glucose, resistin and insulin levels and no significant differences in adiponectin levels compared to the non-treated diabetic group. The effects of metformin were lower in significance than that of date palm seed extract. (figures:1,2,3 and 4).

Table (1) chemical composition of palm date seeds used in the study

Content	*Con.*
Protein %	5.9
Lipid %	4.6
CHO %	25.9
Zn (ppm)	40.6
Mg (ppm)	55.6
K (ppm)	689.3
Ca (ppm)	63.9

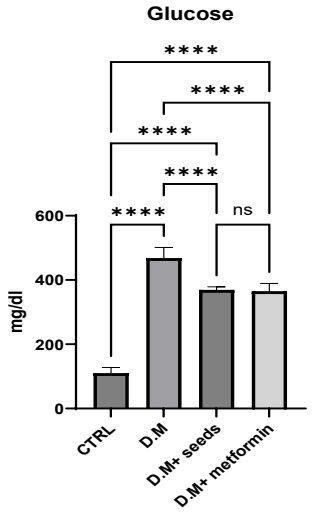

Figure 1: show hyperglycemic condition in diabetic rats while treatments by seeds extract and metformin show decreasing in glucose level in serum, seeds extract show the same effect of metformin in action.

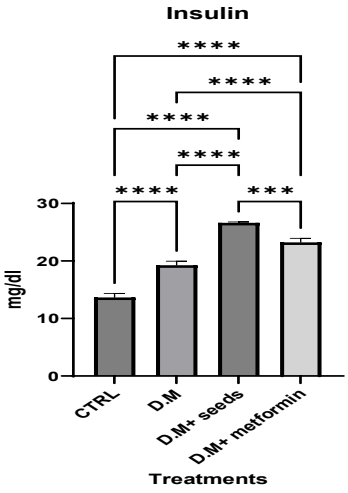

Figure 2: diabetic rats show limited ability to elevate insulin level in blood while treatments by seeds extract and metformin show increasing, seeds extract was the highest show increase in concentration of insulin more than metformin

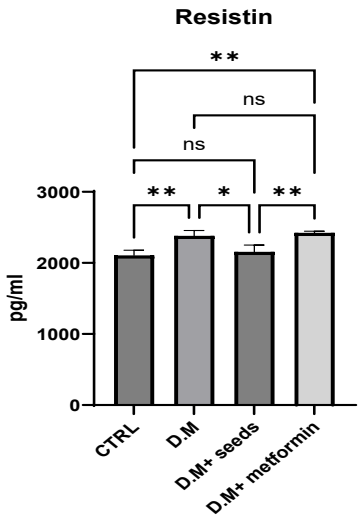

Figure 3: show increasing in Resistin diabetic rats. seeds extract show decreasing, while metformin had no significant alteration in comparsion to D.M.

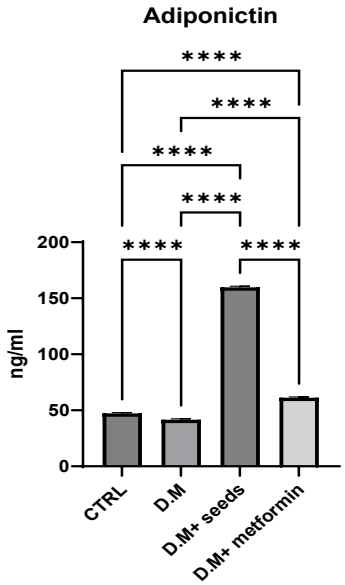

Figure 4:diabetic rats have low level of adiponictin. Seeds extract has ability to enhance adiponictin more than metformin

- Histological results

Alloxan exhibited detrimental effects on islets of Langerhans represented by necrosis and atrophy in the beta cells and the glandular serous cells of the islets, in contrast the date palm seed extract demonstrated the ability to protect pancreatic tissue from damages induced by alloxan when compared to both the non-treated diabetic group and the metformin group.

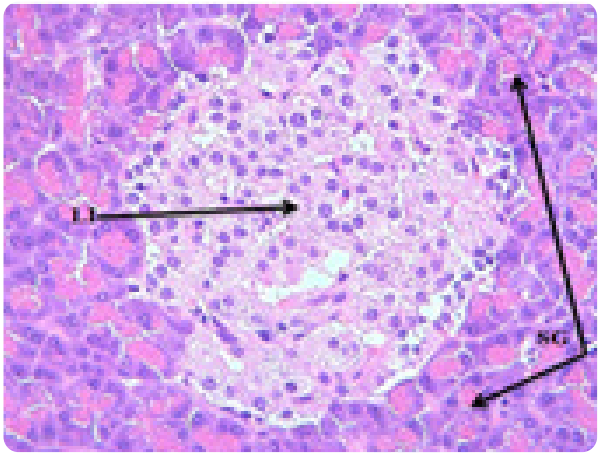

Figure 5: Section of control group pancreas showing islets of Langerhans (LI) and pancreatic serous glands (SG).H&E 400X,

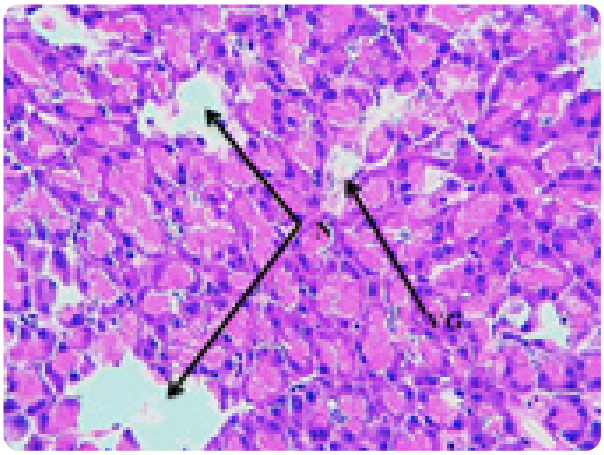

Figure 6: Section of pancreas from alloxan-non-treated group showing tissue necrosis in pancreatic tissue (N) with cellular rupture of serous glands (VG). H & E 400X,

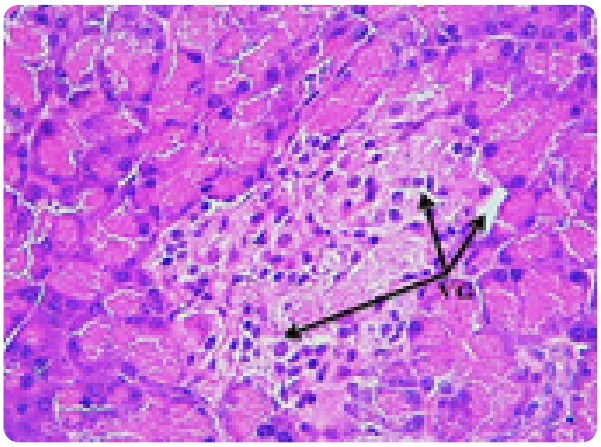

Figure 7: Section of pancreas from alloxan and metformin treated group showing rupture of the islets of Langerhans (VG) and pancreatic serous glands H&E 400X,

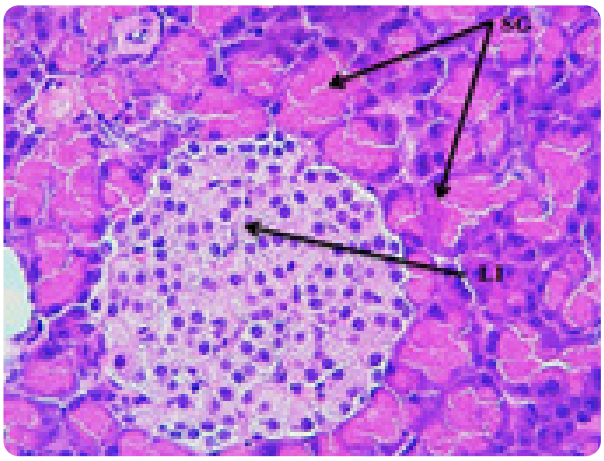

Figure 8: Section of the pancreas of the date seeds aqueous extract group showing the islets of Langerhans (LI) and pancreatic serous glands (SG) in near-normal H&E 400X,

Discussion

Alloxan can enter beta cell via glucose transporter 2 GLUT2 and generates reactive oxygen species ROS and free radicals leading to oxidative stress and cellular damage (20). Free radicals damage cellular membrane, DNA and Proteins leading to beta cell necrosis (21). destruction of beta cell reducing insulin production and causes hyperglycemia (figure:1&2). In non-beta cells of pancreas Alloxan has a weak effect because of lack of GLUT2 expression in these tissues (22).

Palm date seeds have a nutritional value (23). seeds contain carbohydrates, proteins and lipids in addition to vitamins, minerals, phenolic and flavonoids compounds (Table 1), therefore seeds have antioxidant and anti-inflammatory activities. Scientific studies suggested the anti-diabetic effect of palm date seeds extract (24). Polyphenols and flavonoids reduce oxidative stress, inflammation and rehabilitee tissue due neutralizing free radicals(25).

Seeds extract reduce blood glucose (fig.1) inhibit enzymes responsible for breaking down carbohydrate into glucose such as α- amylase and α- glycosidase (26). Also, date seeds can enhance insulin receptor sensitivity and decreasing insulin resistance in addition to improve beta cell to product insulin (27).

Resistin has a pivotal role in D.M. through impairs insulin action and promotes insulin resistance by activating inflammatory mediators such as NF-kB pathway. It also increases enzymes involve in gluconeogenesis (28).Seeds extract show ability to reduce resistin level (fig.3) leading to improve insulin action (fig.2) due decreasing inflammatory pathways, also decreasing gluconeogenesis process (29).

Low adiponectin level is linked to hyperglycemia, insulin resistance and lipid dysfunction. Adiponectin has a vital role in glucose metabolism and insulin sensitivity (30). Adiponectin enhances glucose uptake by skeletal muscle and fatty acid oxidation, also it is stimulating AMP – activated protein kinase in muscle and liver (31). The natural compounds in palm date seeds have a vital role to increase adiponectin expression (fig.4) suppress oxidative

stress and improve insulin signaling . Studies suggested that date seeds may increase Peroxisome proliferator- Activated receptor gamma PPAR-γ that improve adipocyte differentiation and increase level of adiponectin (32). Palm date seeds extract demonstrated a better effect in increasing levels of insulin and adiponectin and decreasing levels of resistin more than metformin while no significant change observed in glucose levels between them as illustrated in figures 1,2,3 and 4.

Conclusion

Palm date seeds extract appear to improve insulin and adiponectin levels through the antioxidant, anti-inflammatory activity and insulin sensitizing effects along with decrease glucose and resistin levels. These findings make seeds extract a promising nutraceutical to improve metabolism and management diabetes. However more studies are required to confirm these effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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