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RESEARCH ARTICLE

Potential antidiabetic effect of date extracts (*Phoenix dactylifera* L.) in Streptozotocin-induced diabetic rats

Hadjer Chenini-Bendiab[1], Noureddine Djebli[1], Meltem Uçar[2]*, Sevgi Kolayli[3]

[1]Laboratory of Pharmacognosy & Api-Phytotherapy (LPAP), Université Abdelhamid Ibn Badis de Mostaganem, 27000 Mostaganem, Algeria, [2]Department of Medical Laboratory Technique, Vocational School of Health Services, European University of Lefke, Lefke, Northern Cyprus, TR-10 Mersin, Türkiye, [3]Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080 Trabzon, Türkiye.

ABSTRACT

For many years, medicinal plants have been used to treat diabetes because they cause less undesirable effects than synthetic drugs. This research aimed to study the *in vivo* antidiabetic activity of Algerian date pulp of the Deglet Noor variety in streptozotocin (STZ)-induced experimental diabetes. The polyphenol composition was established by RP-HPLC-UV and the sugar contents were investigated by HPLC-RID of the ethanolic extract of date pulp. Aqueous date pulp extracts (AP) was administrated to STZ (60 mg/kg b.w.) induced diabetic rats at doses of 150 and 300 mg/kg b.w. (APD1 and APD2) for four weeks. Glibenclamide (5 mg/kg b.w.) was chosen as a reference treatment. The administration of APD1 reduced significantly ($p<0.01$) fasting blood glucose levels in diabetic rats and also moderately restored body weight as compared to the diabetic control group. A significant decrease in plasma fasting blood glucose and serum lipid profile Total Cholesterol and LDL was observed after administration of APD1 and APD2 in diabetic groups compared with the diabetic control group. Diabetic treated rats received APD1 and APD2 have shown a beneficial treatment response against diabetic complications in pancreas, liver and kidney tissues. This study suggests that the aqueous Algerian date pulp extracts of the Deglet Noor variety have a potential antidiabetic effect by decreasing fasting blood glucose and serum lipid levels and preventing pancreas, liver and kidney tissue of diabetic rats that could be due to its rich composition of active compounds.

Keywords: Antidiabetic activity; Phenolic compounds; *Phoenix dactylifera* L.

INTRODUCTION

Diabetes mellitus (DM) is a serious health scourge that has attained alarming dimensions; it is a real health threat, which does not depend on socioeconomic status and knows no borders. In 2019, International Diabetes Federation estimated 463 million the number of people living with diabetes and this number is expected to reach 700 million in 2045 (Saeedi et al., 2019). Diabetes is a chronic metabolic disturbance characterised by hyperglycemia, causing deficiencies in carbohydrate, lipid and protein metabolism(Soni et al., 2018). This dysfunction occurs when the body does not produce enough insulin (Insulin-dependent DM) or cannot effectively use the insulin it produces (Noninsulin-dependent DM). If not controlled in the long term, insulin deficiency could cause disabling complications and potentially fatal (Forbes and Cooper, 2013). Nowadays, several synthetic drugs are used for the treatment of diabetes, including insulin therapy or synthetic oral hypoglycemic agents. However, the use of these drugs is not harmless, and their toxicity can lead to organ lesions, such as liver and kidney disorders, skin rash, edema, dizziness, upset stomach, abdominal pain and bloating, diarrhea, tiredness which limit their applications (Khaliq et al., 2016). Currently, scientific research is directed towards new natural therapeutic sources, thus resulting in products possessing antidiabetic properties with less toxicity (Soni et al., 2018). It is distinguished mainly by its nutritional values and its sources of energy, but not only, the date fruit (*Phoenix dactylifera* L.) also has interesting therapeutic properties, such as antioxidant, antimicrobial, gastroprotective, immunostimulant, anticancer and anti-inflammatory effects (El-Far et al., 2019). Recent phytochemical investigations have shown that dates contain several bioactive compounds, which give them pharmacological activities. Indeed, this fruit is a rich source of phenolics, anthocyanins, proanthocyanidins, sterols, carotenoids, and flavonoids but its composition varies

***Corresponding author:** Meltem Uçar, Department of Medical Laboratory Technique, Vocational School of Health Services, European University of Lefke, Lefke, Northern Cyprus, TR-10 Mersin, Turkey. **Phone:** +90 392 6602000-2587, **Cellular Phone:** +90 533 8382624, **Fax:** +90 392 7277528. **E-mail:** mucar@eul.edu.tr

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depending on the types, maturity, geography, and climate where they are collected (El-Far et al., 2019, Hussain et al., 2020, Mia et al., 2020). Previous researchs have reparted that these active constituents play a role in free radical scavenging via antioxidant activities and by inhibiting α-amylase and α-glucosidase enzymes (Eddine et al., 2014, Farag et al., 2015, El Abed et al., 2017, Moqsood et al., 2020). Based on this scientific orientation, the aim of this research was to determine the phenolic profile and the sugar contents of Algerian date pulp extracts of the Deglet Noor variety, and for the first time to investigate its antidiabetic potential and protective role against diabetic complications through *in vivo* experiments with STZ-induced diabetic rats.

MATERIAL AND METHODS

Chemicals

Streptozotocin(STZ), phenolic standards, sugar standards, ethanol, diethyl ether, ethyl acetate, acetonitrile, acetic acid, methanol, sodium citrate, 10% formalin solution, xylene, haematoxylin and eosin were provided from Sigma-Aldrich (St. Louis, MO, USA). Glibenclamide was procured from Hikma Pharma Algeria Laboratory.

Experimental design

Phenolic, flavonoid profil and sugar contents of ethanolic Algerian date pulp extracts were determined by RP-HPLC-UV and a refractive detector (RID) with HPLC. *In vivo* antidiabetic activity of aqueous extracts of Algerian date fruit investigated Streptozotocin induced rats by biochemical analyses and histological examination of tissues. Experimental design were shown in Fig. 1.

Plant material and extracts preparation

Date palm fruit of Deglet Noor variety was collected from Biskra, Algeria. Date pulps were crushed into small pieces to obtain a paste. Alcoholic extract of the sample was obtained by mixing with ethanol (1:4, w/v) for 24h at room temperature (25±5°C). The aqueous extract was subjected to a maceration process with distillate water (1:10, w/v) at 4°C for 48h (Al-Qarawi et al., 2008). Solids were separated by centrifugation at 1000 rpm for 10 min and then filtration according to the protocol of Al-Farsi and Lee, (2008).

Phenolic and flavonoid profile

Ethanolic date pulp extract (EP) intended for phenolic profile analysis was prepared according to the protocol described by Chenini-Bendiab et al., (2021). Determination of phenolic acids and flavonoids in the extract studied was carried out by reversed-phase high-performance liquid chromatography and ultraviolet spectrometry (RP-HPLC-UV) (Elite LaChrom Hitachi, Japan) according to the protocol of Can et al., (2015).

Sugar contents analysis

Sugar analysis of date extract was performed using a refraction detector (RID) with HPLC (Elite LaChrom, Hitachi, Japan) following the protocol established by Can et al., (2015). The calibration graph of each sugar was obtained and the results % values of mono-, di- and trisaccharides per 100 g of FW.

Antidiabetic activity

Animals

Male Wistar rats weighing 200-250 g were provided by the Pasteur Institute of Algiers- Algeria. Animals were kept under standard conditions of temperature and humidity

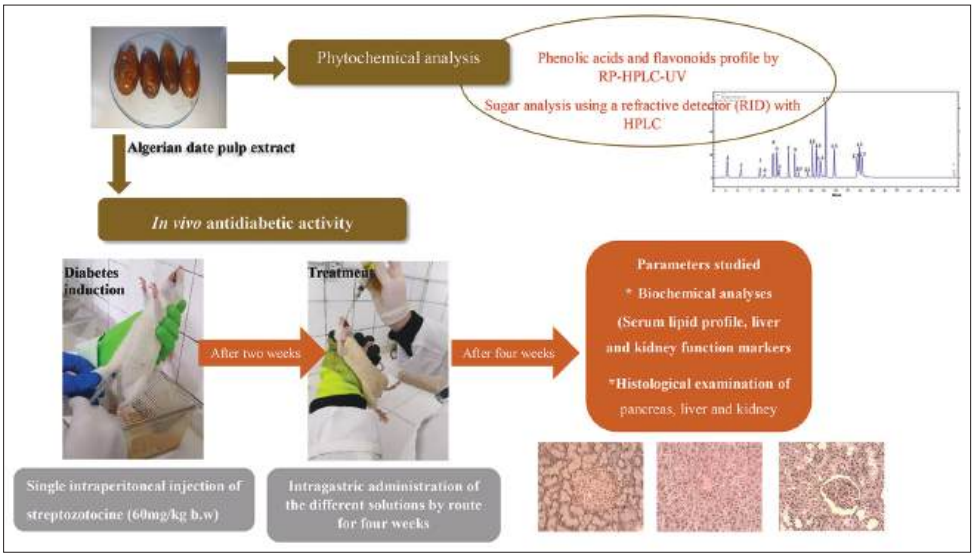

Fig 1. General diagram of the experiments.

with alternating 12h light and dark cycles. They had free access to standard food and water *add libitum*. The experimental protocol was agreed in accordance with the recommendations established by the Pharmacognosy and Apiphytotherapy Laboratory (University of Mostaganem), following the instructions mentioned by the Institutional Animal Care and Use Committee (IACUC) (Code:01/SNV/21).

Acute toxicity test

The acute toxicity of aqueous extract of date pulp (AP) was carried out in accordance with OECD standards, 425 guidelines, 2008. Thirty Wistar rats were divided into three groups (n=10). Each group received AP extract intragastrically at increasing doses of 150, 300 and 1000 mg/kg b.w. All animals were observed for behavioral and neurological effects at 24h until 14 days. The death rate was registered the whole length of this period, the lethal dose (LD50) was calculated in rats using the method of Abdel-Barry et al., (1997).

Experimental diabetes induction

Diabetes was caused after overnight fasting in rats (n=28) by a single intraperitoneal injection of streptozotocin (60 mg/kg b.w.) (Furman, 2015). Sucrose solution (10%) was given immediately for 24h to avoid hypoglycemia. The control group (n=7) received only vehicle (sodium citrate buffer). After 72 hours, hyperglycemia was confirmed in the STZ-induced rats (fasting glycaemia >250 mg/dl). A period of two weeks was established before starting treatment, to maintain and stabilize the hyperglycaemic state in rats (Adeyemi et al., 2010).

Rats were divided into five groups (seven animals each): Group 1: Control (C) received water. Group 2: Diabetic control (DC) received water. Group 3 and 4: Diabetic treated rats received AP at 150 mg/kg b.w. (D-APD1) and 300 mg/kg b.w. (D-APD2) respectively. Group 5: Diabetic treated rats received a standard drug Glibenclamide at 5 mg/kg b.w. (D-STD). The different solutions were administered by intragastric route for 4 weeks. After this treatment period, two weeks were extended, in order to observe the evolution of the analyzed parameters.

Body weight and solution consumption measurement

The weight evolution (g) of each rat was estimated once a week throughout the experiment period. While water consumption (ml) was measured daily for each group.

Blood glucose analysis

Fasting blood glucose was measured weekly throughout the experimentation period (8 weeks) using a digital glucometer (Vital Check, MM1200. TECO diagnostics, USA). After eight weeks rats were sacrificed and central blood was obtained. Fasting plasma blood glucose was determined by the colorimetric enzymatic technique of Trinder method (Trinder, 1969).

Biochemical parameters studied

Serum lipid profiles, liver and kidney functions markers were measured using an automated biochemical analyser (EliTechGroup, Clinical Système. Selectra PROM. Netherlands).

Histological examination

After sacrifice of the animals, the pancreas, liver and kidneys were surgically excised and fixed in 10% formalin. Tissues were cut at 4μm using the microtome and then stained with haematoxylin and eosin following the technique established by Merck, (2010).

Statistical analysis

All the results were expressed as mean ± SEM. Results were analyzed using one-way ANOVA test followed by Turkey's multiple comparison tests.

RESULTS

Phenolic profile

Nineteen phenolic standards were identified and quantified using RP-HPLC-UV. Gallic, protocatechuic, p-OH benzoic, syringic, p-coumaric, ferulic and t-cinnamic acids were present in EP, while caffeic acid was not detected. About flavonoid contents: Chrysin, epicatechin and catechin marked a significant amount in EP extract. Whereas, rutin, myricetin, resveratrol, luteolin and hesperetin were not identified (Table 1).

Sugar contents

Analysis of date sugar was performed with HPLC-RID and eight sugar standards were analyzed. Reducing sugars in the form of glucose and fructose found in date pulp were 16,99 and 13,08% respectively. While sucrose as a non-reducing sugar recorded a higher level 38,44%. However, ribose, maltose, trehalose, melibiose, melezitose were not detected (Table 2).

Acute toxicity

The results of acute toxicity showed no mortality and any behavioral disorder following 14 days post-administration of the AP at 150, 300 and 1000 mg/kg b.w.

Table 1: Total phenolic compounds of ethanolic date pulp extract (EP) by RP-HPLC-UV (mg/g FW)

Standards	EP
Gallic Acid	11.013
Protocatechuic acid	3.213
p-OH Benzoic acid	2.928
Catechin	4.739
Caffeic acid	N.D.
Syringic acid	1.378
Epicatechin	8.054
p-Coumaric acid	6.282
Ferulic acid	9.375
Rutin	N.D.
Myricetin	N.D.
Resveratrol	N.D.
Daidzein	3.008
Luteolin	N.D.
t-Cinnamic acid	0.559
Hesperetin	N.D.
Chrysin	29.748
Pinocembrin	3.655
CAPE	5.862

EP: Ethanolic date pulp extract. N.D.: Note detected.

Table 2: Sugar contents of ethanolic date pulp extract (EP) by HPLC-RID (%/100g FW)

Sugars	EP %
Ribose	N.D.
Fructose	13.08
Glucose	16.99
Sucrose	38.44
Maltose	N.D.
Trehalose	N.D.
Melebioz	N.D.
Melezitose	N.D.

EP: Ethanolic date pulp extract. N.D.: Note detected.

Antidiabetic activity

Effect of AP on body weight and water consumption

The weight change observed in the control group showed regular growth (42.77%) throughout the experiment. However, all diabetic groups recorded a very significant ($p<0.001$) decrease in body weight change p. Nevertheless, the diabetic treated groups (D-APD1, D-APD2 and D-STD) showed a moderate decrease in body weight change as compared to DC (Table 3).

The volume of water consumed by the diabetic groups after STZ administration was significantly higher ($p<0.001$) than the control group. Whereas, D-APD1 group showed significantly ($p<0.01$) low consumption compared to DC during the treatment period (Table 4).

Table 3: Effect of AP on body weight in STZ-induced diabetic rats. Data represented as mean ± SEM (n=7)

Groups	Body Weight (g)		
	Initial	Final	% Change
C	211.29±11.60	301.67±14.44	42.77
DC	236.33±4.97	174.00±9.25***	-26.37
D-APD1	203.50±11.81	175.25±20.67***	-13.88
D-APD2	209.00±12.43	176.00±19.57***	-15.79
D-STD	212.00±21.21	188.67±19.81***	-11.01

AP: Aqueous date pulp extracts, C: Control Group, DC: Diabetic Control Group, D-APD1: Diabetic Treated Rat Group received AP at 150 mg/kg, D-APD2: Diabetic Treated Rat Group received AP at 300 mg/kg, D-STD: Diabetic Treated Rat Group received glibenclamide at 5 mg/kg. ***p <0.001 compared with the control group.

Table 4: Effect of AP on water consumption in STZ-induced diabetic rats. Data represented as means ± SEM (n=7)

Groups	Water Consumption (ml)		
	Before Treatment	During Treatment	After Treatment
C	182.45±1.84	171.46±15.06	213.93±10.61
DC	656.41±25.68***	743.89±34.81***	875.43±7.48***
D-APD1	598.02±10.93***	692.46±40.81***##	840.50±26.77***
D-APD2	680.30±63.01***	774.25±45.17***	846.36±27.98***
D-STD	691.07 41.11***	732.36±42.56***	778.14±3.64***#

AP: Aqueous date pulp extracts, C: Control Group, DC: Diabetic Control Group, D-APD1: Diabetic Treated Rat Group received AP at 150 mg/kg, D-APD2: Diabetic Treated Rat Group received AP at 300 mg/kg, D-STD: Diabetic Treated Rat Group received glibenclamide at 5 mg/kg. ***p <0.001 compared with the control group, #p<0.05 and ##p<0.01 compared to diabetic control group.

Effect of AP on blood glucose

After 72 hours of the STZ administration, the diabetic groups recorded a highly significant ($p<0.001$) sharp increase in fasting blood glucose level compared to the control group. Nevertheless, D-APD1 group demosnstrated a highly significant decrease in fasting blood glucose during ($p<0.001$) and after treatment ($p<0.01$) compared to DC. Treatment with APd1 did not completely normalise blood glucose levels. Fasting plasma blood glucose measured after 8 weeks of the experiment showed a highly significant ($p<0.001$) increase in DC and diabetics treated with glibenclamide (D-STD). The administration of APD1 and APD2 induced in diabetic rats a significant ($p<0.05$) decrease in plasma blood glucose compared to DC, without restoring healty blood glucose (Table 5).

Effect of AP serum lipid profile

The diabetic control group showed a significant increase in serum TC ($p<0.05$), while the elevation of HDL and LDL levels was not significant compared to the control group. However, TC and LDL levels in the D-APD1 and D-APD2 groups were found to be significantly ($p<0.05$) reduced as compared to the DC (Table 6).

Table 5: Effect of AP on fasting blood glucose during experimentation. Data represented as means ± SEM (n=7)

Groups	Fasting Blood Glucose Level (mg/dl)			
	Before Treatment	During Treatment	After Treatment (72h)	After Treatment (8 Weeks)
C	126.10±10.02	129.89±10.26	133.67±6.47	240.00±37.00
DC	411.56±149.81***	397.45±106.76***	426.13±102.36***	515.00±19.00***
D-APD1	368.53±138.70***	278.15±99.10***	306.85±119.17***	375.00±6.00*#
D-APD2	393.66±136.90***	365.19±92.10***	384.63±102.23***	384.00 85.00*#
D-STD	382.08±117.43***	338.31±106.20***	457.00±77.70***	551.50±12.50***

AP: Aqueous date pulp extracts, C: Control Group, DC: Diabetic Control Group, D-APD1: Diabetic Treated Rat Group received AP at 150 mg/kg, D-APD2: Diabetic Treated Rat Group received AP at 300 mg/kg, D-STD: Diabetic Treated Rat Group received glibenclamide at 5 mg/kg. ***p <0.001 and *p <0.05 compared with the control group, #p<0.05 compared to diabetic control group.

Table 6: Effect of AP on serum TC, TG, HDL, LDL in STZ-induced diabetic rats. Data represented as means ± SEM (n=7)

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
C	51.33±4.04	45.33±11.68	19.67±0.58	23.00±1.73
DC	70.50±9.50*	43.50±16.50	27.00±5.20	34.50±3.50
D-APD1	49.50±4.50#	41.50±3.50	23.00±3.00	18.00±1.00##
D-APD2	47.67±8.14#	43.00±11.14	22.33±8.08	17.00±7.81##
D-STD	60.00±5.57	52.00±9.54	25.67±2.08	23.67±5.13

AP: Aqueous date pulp extract, C: Control Group, DC: Diabetic Control Group, D-APD1: Diabetic Treated Rat Group received AP at 150 mg/kg, D-APD2: Diabetic Treated Rat Group received AP at 300 mg/kg, D-STD: Diabetic Treated Rat Group received glibenclamide at 5 mg/kg, TC: Total Cholesterol, TG: Triglycerides, HDL: High-density lipoproteins, LDL: Low-density lipoproteins. *p < 0.05 compared to control group. #p < 0.05, ##p < 0.01 compared to diabetic control group.

Table 7: Effect of AP on serum AST, ALT, Urea and Creatinine in STZ-induced diabetic rats. Data represented as means ± SEM (n=7)

Groups	AST (UI/L)	ALT (UI/L)	Urea (mg/dl)	Creatinine (mg/dl)
C	77.00±3.61	40.00±7.00	29.33±4.51	6.47±0.09
DC	69.33±11.36	88.00±7.81**	71.00±17.52**	5.84±0.14*
D-APD1	78.50±26.50	54.00±22.61	43.33±5.03#	4.99±0.09***#
D-APD2	109.33±26.69	70.67±17.21	71.33±8.02**	5.80±0.38
D-STD	94.00±36.04	67.00±7.00	61.33±9.87*	6.16±0.57

AP: Aqueous date pulp extracts, C: Control Group, DC: Diabetic Control Group, D-APD1: Diabetic Treated Rat Group received AP at 150 mg/kg, D-APD2: Diabetic Treated Rat Group received AP at 300 mg/kg, D-STD: Diabetic Treated Rat Group received glibenclamide at 5 mg/kg, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. *p<0.05, **p<0.01, ***p<0.001 compared to control group. #p<0.05 compared to diabetic control group.

Effect of AP on liver and kidney function markers

Serum AST and ALT levels were found to be high in diabetic groups compared with the control group. The elevation of ALT was significant ($p<0.01$) in the DC. Serum urea level recorded significantly ($p<0.01$) higher values in DC, D-APD2 and D-STD. However, D-APD1 group showed a significant ($p<0.05$) decrease in this parameter compared to the DC. Moreover, serum creatinine level was found to be significantly reduced in D-APD1 group as compared to the DC and C (Table 7).

Histological study

Histology of pancreatic tissue in the healthy group showed a regular architectural appearance. Normal dispersion in size and number of Langerhans islets was found within the acini. Unlike DC, which showed a significant reduction in the proportion of islets with severe hypertrophy. A ramification of the polygonal shape of the glands with irregularity of outlines as well as a rarefication of the endocrine cells was also observed. The treatment with AP extracts at 150 and 300 mg/kg b.w. reduced DM-induced changes in the pancreatic islets. Indeed, the morphology of the islets appeared improved with a regular outline, the number of the endocrine cells was also restored almost as similar as the control group. Whereas, the D-STD group showed a structural aspect of the Langerhans islets almost similar to that found in the diabetic group, corresponding to hypertrophied endocrine glands with irregular outlines and a rarefication of endocrine cells (Fig. 2).

The investigation of liver histology revealed in the control group a normal hepatic architectural aspect in the presence of hepatocytes and hepatic sinusoids that were arranged in plates radiating from the central vein. However, the diabetic group showed a disturbance of the liver lobule. Lipids were accumulated as intracellular macro-vacuoles corresponding to mild steatosis, some apoptotic hepatocytes and dilatation of hepatic sinusoids were also noted. While the diabetic groups treated (D-APD1, D-APD2, D-STD) demonstrated a more or less similar hepatic lobule aspect to the control group, except some dilatation of sinusoids and activation of Kupffer cells (Fig. 3).

Histology of kidney tissue of the healty group demonstrated a regular architectural structure of the glomerulus surrounded by a regular outline of Bowman's capsule. The histological aspect of the kidney in treated diabetic groups (D-APD1, D-APD2, D-STD) showed apparent similarities compared to the control group. Indeed, the absence of diabetic nephropathy signs was noted, moreover, no diffuse and nodular mesangial expansion was observed. However, shrinkage glomerulus with heterogeneous architecture and disintegration of Bowman's capsule was found in the diabetic control group (Fig. 4).

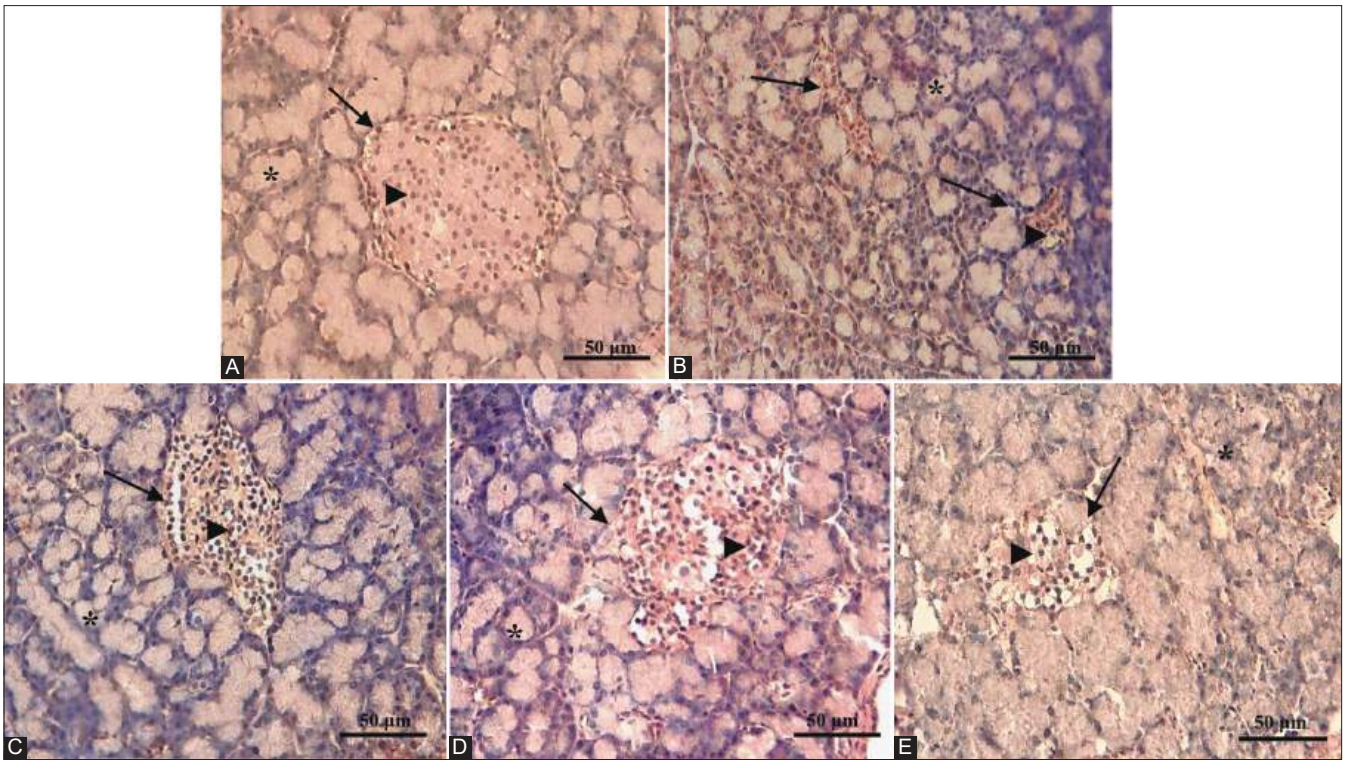

Fig 2. Photomicrograph of pancreatic tissue stained with Haematoxylin and Eosin (X40). A: Control group shows dense-staining acini (asterisk) and a light-staining normal islet of Langerhans (arrow) clogged with endocrine cells (arrowhead). B: Diabetic control group (DC) shows hypertrophy of pancreatic islets (arrows) with a rarefication of endocrine cells (arrowhead). C: Diabetic rats treated with aqueous date pulp extract at 150 (D-ADP1). D: Diabetic rats treated with aqueous date pulp extract at 300 mg/kg (D-APD2). C and D respectively show an improvement in size and number of Langerhans islets (arrows) and an increase in the number of endocrine cells (arrowhead). E: Diabetic rats treated with glibenclamide at 5mg/kg (D-STD) shows a heterogeneous architectural aspect of pancreatic islets (arrows) with rarefication of endocrine cells (arrowhead). Scale Bars= 50µm.

DISCUSSION

The mechanism of antidiabetic treatment is to increase the rate of regeneration of pancreatic beta cells, enhance the secretion and synthesis of insulin and reduce the resistance of target cells to insulin. (Hasan and Mohieldein, 2016). In this study, the phytochemical analysis was intended to detect phenolic substances and sugar contents of EP. The results of RP-HPLC-UV analysis have demonstrated the presence of most phenolic compounds studied with different concentrations. Almost similar contents of phenolic acid and flavonoids have been reported by Benmeddour et al., (2013) in aqueous acetone extract of Algerian date and Bouhlali et al., (2018) in Moroccan-date methanolic aqueous extract. Also, phenolic compositions of a date varies according to a numner of factors, including variety, stage of maturity and climatic conditions (Hussain et al., 2020).

Dates are known for their high level of sugars, which are considered the source of energy and nutrition. The sugars identified of EP were in agreement with those published by Hamad et al., 2015 who found that most of the studied cultivars on Saudi Arabian date had more glucose and fructose than sucrose except Nabtit Ali, Sokary and Rashodia cultivars which had higher concentrations of sucrose.

This experiment aimed to highlight the antidiabetic activity of AP and consequently, to determine a potential hypolipidemic effect and a likely restoration of liver and kidney functions due to diabetic complications. This assessment was based on STZ-induced diabetic rats. The effect of STZ is principally due to the targeted alteration of pancreatic islet-dependent betta cells, leading to diabetes mellitus (Furman, 2015).

Induction of diabetes caused in rats weight loss and high water consumption. The reduction in body weight observed could be explained by the deficiency in carbohydrates for energy metabolism (Hasan and Mohieldein, 2016). A moderate increase in the body weight change of diabetic treated groups (D-APD1, D-APD2, D-STD) compared to the DC signifying the inversion of gluconeogenesis (Abiola et al., 2018).

The treatment with APD1, APD2 and glibenclamide reduced hyperglycaemia in diabetic rats compared to the

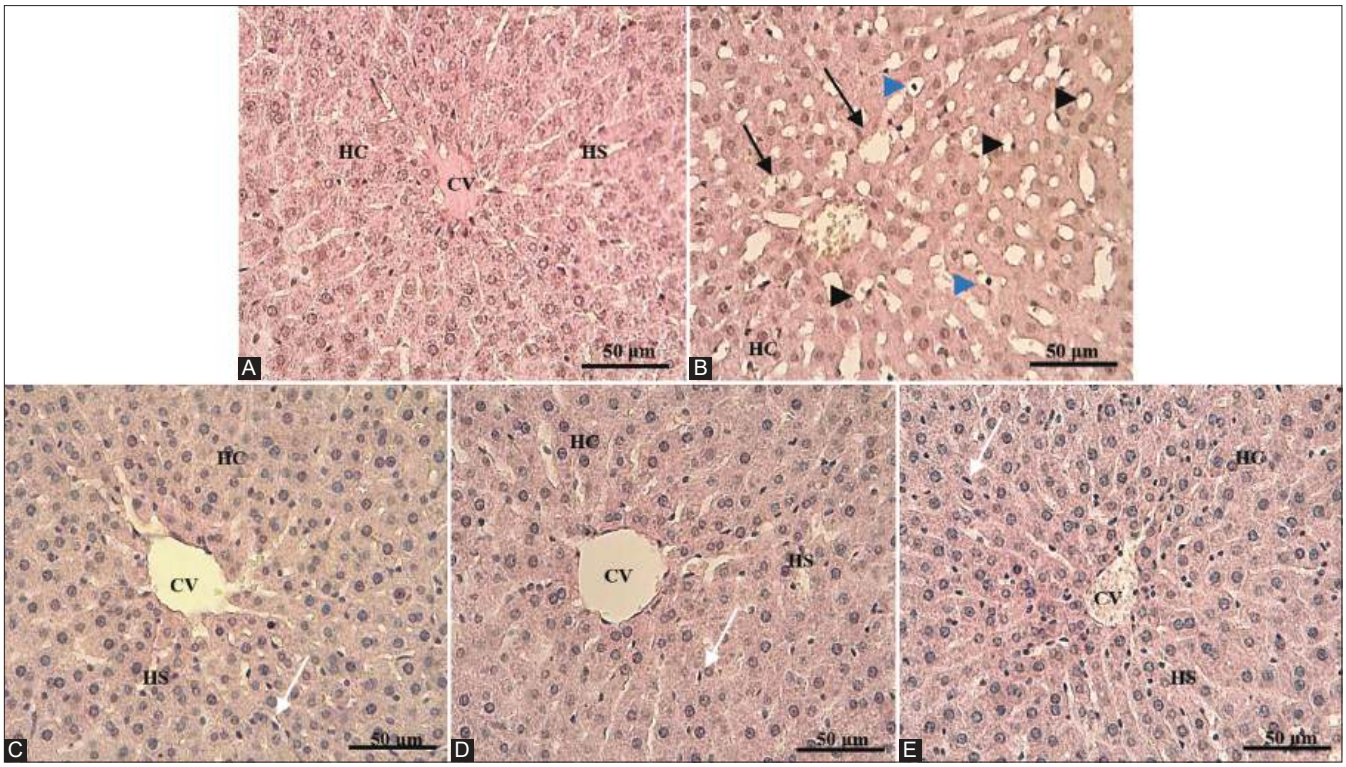

Fig 3. Photomicrograph of hepatic tissue stained with Haematoxylin and Eosin (X40). A: Control group shows a uniform architecture of hepatic lobule centred by the central vein (CV). Normal appearance of hepatocyte spans (HC) separating radiated hepatic sinusoids (HS) converging to the centro-lobular vein. B: Diabetic control group (DC) shows macro-vacuoles (arrowheads) associated with apoptotic hepatocytes (blue arrowhead) and some dilatation of hepatic sinusoids (arrows). C: Diabetic rats treated with aqueous date pulp extract at 150 (D-ADP1). D: Diabetic rats treated with aqueous date pulp extract at 300 mg/kg (D-ADP2). E: Diabetic rats treated with glibenclamide at 5 mg/kg (D-STD). C, D and E respectively show a hepatic lobule more or less similar to control group, except activation of Kupffer cells (white arrows). Scale Bars= 50µm.

DC during treatment. This could be explained by the ability of the AP extract to stimulate pancreatic secretion of insulin from the remaining pancreatic cells. Several previous studies have shown that the anti-diabetic activity could be justified by different hypotheses, such as activation of the antiradical process, strong inhibition of α-amylase and α-glucosidase, or stimulation of glucose absorption metabolism to lead to normal glucose homeostasis (Gray et al., 2000, Khaliq et al., 2016, Echegaray et al., 2020). A similar observation has also been reported by other authors who have inverstigated experimental diabetes (Abiola et al., 2018, Soni et al., 2018). Other studies cited by Hussein et al., (2015) who studied methanolic and aqueous extracts of Egyptian date pulp were in agreement with our results.

However, plasma blood glucose levels demonstrated the hypoglycemic effect, which is extended after 15 days of the end of treatment with APD1 and APD2, unlike glibenclamide, which has an instantaneous effect. This prolonged action could be linked to the capacity of the bioactive substances contained in PA to act at different levels, such as activating insulin production by the remaining betta cells or promoting the regeneration of pancreatic beta cells.

Lipid disorders are usually a secondary complication of diabetes. Usually, the increase in TC, TG and LDL levels and the reduction in HDL levels are generally the consequences of hyperglycaemia due to insulin deficiency. In this study, the results confirmed this approach, since the DC showed an increase in TC and LDL levels compared to the control group. However, the treatment with APD1 and APD2 significantly restored TC and LDL levels compared to DC. This finding is in agreement with the result reported by Soni et al., (2018).

Hyperglycemia leads to hepatic damage resulting in an elevation in serum hepatic markers. The disturbances of ALT and AST levels have been observed in diabetic rats compared with the control group. Hasan and Mohieldein, (2016) Reported similar conclusions in their work. However, the treatment with APD1 resulted in a great ability to restore this elevation in the liver biomarkers almost to normal. This is in accordance with the findings of Abdelaziz et al., (2015).

The present study demonstrated a significant increase in serum urea levels in diabetic rats compared to control. In consistence with this finding, a previous study noted a similar result (Dewanjee et al., 2011). Whereas, the

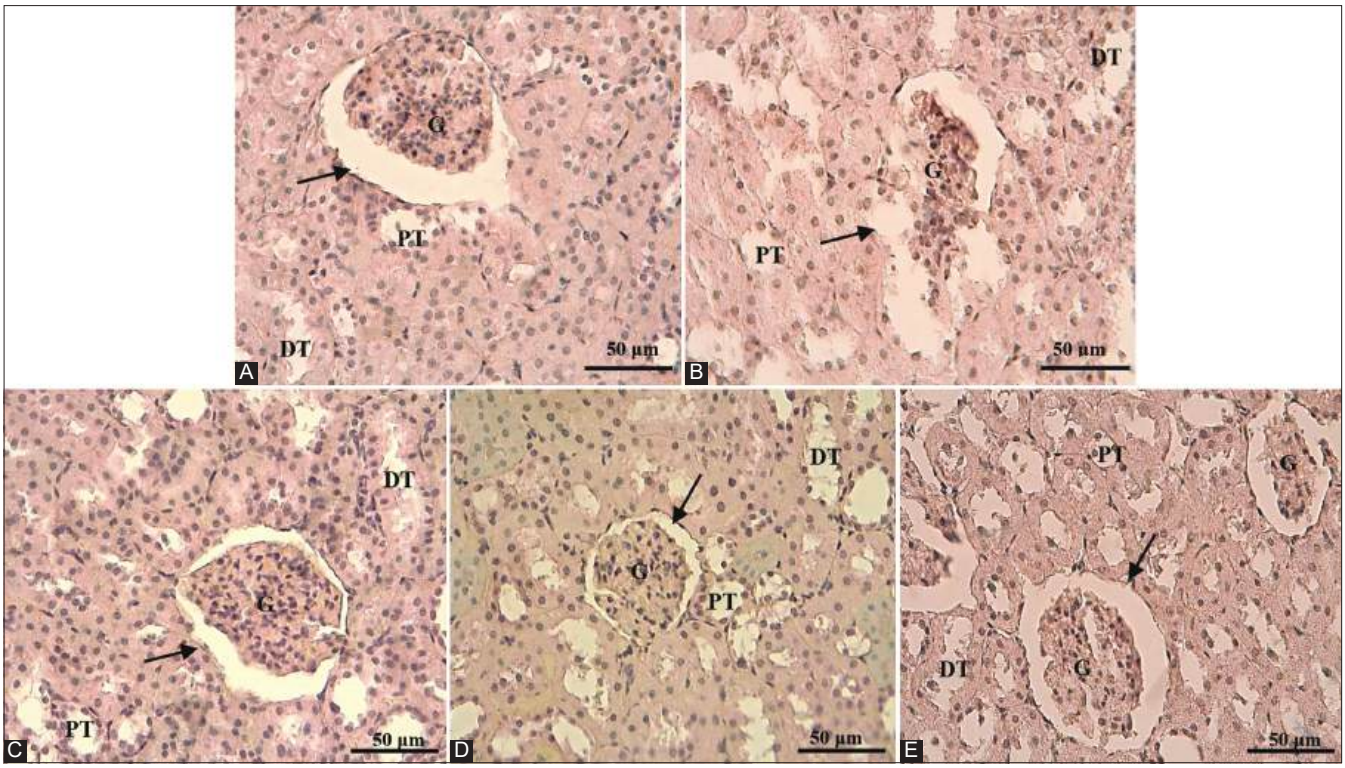

Fig 4. Photomicrograph of kidney tissue stained with Haematoxylin and Eosin (X40). A: Control group shows a normal structure of glomerulus (G) and regular Broun's capsule outlines (arrow), proximal convoluted tubules (PT) and distal convoluted tubules (DT) appear with normal thickness. B: Diabetic control group (DC) shows shrinkage glomerulus (G) and disintegration of Bowman's capsule (arrow). C: Diabetic rats treated with aqueous date pulp extract at 150 (D-ADP1). D: Diabetic rats treated with aqueous date pulp extract at 300 mg/kg (D-APD2). E: Diabetic rats treated with glibenclamide at 5 mg/kg (D-STD). C, D and E respectively show an almost normal architectural structure of glomerulus (G) with regular Broun's capsule outlines (arrow). Scale Bars= 50µm.

treatment with APD1 significantly reduced serum urea and creatinine levels in diabetic rats compared to DC. This result is in agreement with those reported by Al-Qarawi et al., (2008) who found that the treatment with Flesh palm date lead a significant decrease in serum creatinine and urea concentrations in gentamicin-induced nephrotoxicity in rats. Other results also showed that treatment with aqueous and methanolic extracts of date palm fruit led to a significant improvement in kidney function by reducing serum creatinine, urea and uric acid (El Mousalamy et al., 2016). El Arem et al., (2014) reported the nephroprotective effect of aqueous date extracts against dichloraacetic acid-induced kidney damage.

Histological examination of pancreatic tissue approved earlier results. Microscopic observation of pancreatic islet revealed several damage caused by STZ in the DC. Although the cytotoxic action of beta cells by STZ is not clearly elucidated. It is probably linked to DNA methylation caused by this diabetic agent. STZ may be inhibiting the free radical scavenging enzyme, thus enhancing superoxide production, which is responsible for DNA damage (Spinas, 1999). However, treatment of APD1 and APD2 restored STZ- induced lesions in the pancreatic islets, which explains the decrease in blood sugar levels during and after treatment. These actions could be explained by the bioactive compounds contained in date pulp extract such as polyphenols known for their antioxidant effect, this property consists of inhibiting lipid peroxidation and NO generation (Abdelaziz et al., 2015, Echegaray et al., 2020). Results in this study are in agreement with previous reports (Khaliq et al., 2016, Melek et al., 2019). Treatment with glibenclamide did not improve pancreatic cells in diabetic rats, confirming that it primarily affects the activation of insulin secretion and not the regeneration of beta cells. This observation is not consistent with the results reported by Ahmed et al., (2010), who noted a reactivation of β-cells by glibeclamide in diabetic rats treated with alloxane.

Histological results of liver tissue demonstrated a disturbance in the normal architecture of hepatic lobules with mild steatosis in DC. The treatments of APD1 and APD2 have improved the morphological structure of the liver by reducing the changes induced by diabetes, which is consistent with the previously reported finding (Melek et al., 2019).

Histological analysis of kidneys obtained from DC revealed the heterogeneous architecture of glomerulus without

glomerular sclerosis and mesangial expansion. This could be explained by acute renal impairment, unlike Melek et al., 2019 who have noted severe glomerular damage related to chronic diabetic nephropathy. However, treated diabetic rats (D-APD1, D-APD2, D-STD) showed a similar morphological aspect to the control group, probably because acute renal impairment was not accompanied by severe glomerular destruction, pathognomonic of chronic kidney nephropathy requiring more time. Previous studies have reported similar findings, such as significant increase in glomerular damage and loss of tubular epithelium (El-Mousalamy et al., 2016.)

CONCLUSION

This study established the positive role played by Algerian date pulp extract (Phoenix dactylifera L.) in the antidiabetic activity of experimental diabetes. But not only that, since treatment with our study sample also let to attenuation of damage to the pancreas, liver and kidneys. These results have made it possible to attribute for the first time to the Deglet Noor variety of date fruit the efficacy of treating hyperglycaemia and associated complications, which could constitute a potential treatment for diabetes in the future. Further research will be wssential to identify the exact phytochemicals employed and their mode of action from a molecular and cellular ponint of view in the therapeutic process against diabete.

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Conflict of interest

The authors declare no conflict of interest in this study.

Author's contributions

Hadher Chenini-Bendiab: Conceptualization, Methodology, Data collection, Sample analysis, Data analysis, Writhing the initial draft; Noureddine Djebli: Conceptualization, Methodology, Data analysis, Student supervision, Project leadership, Project management; Sevgi Kolayli: Sample analysis, Meltem Uçar: Writing the initial draft and revisions.

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