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Original Article

Antioxidant properties of date seeds extract (*Phoenix dactylifera* L.) in alloxan induced damage in rats

Propriedades antioxidantes do extrato de sementes de tâmaras (*Phoenix dactylifera* L.) em danos induzidos por aloxana em ratos

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Abstract

The study was conducted to examine the antioxidant activity and evaluate the protective effects of the date seeds powder kentichi against alloxan-induced damage in the liver, kidney, and pancreas in diabetic's rats. Group 1: control group, that did not receive any treatment, Group 2: alloxan was injected intraperitoneally (120 mg/kg body weight) for two days (Diab), Group 3: treated only by date seeds powder added in the diet (300 g/kg) for 6 weeks (DSPK), Group 4: alloxan-diabetic rats treated with date seeds powder (300 g/kg) (DSPK + Diab). Estimations of biochemical parameters in blood were determined. TBARS, SOD, CAT, and GPx activities were determined. A histopathological study was done by immersing pieces of both organs in a fixative solution followed by paraffin hematoxylin-eosin staining. In addition, the antioxidant activities of DSPK were evaluated by DPPH radical scavenging activity, reducing power, and ABTS free radical scavenging. The results revealed that date seeds significantly decreased serum levels of glucose, cholesterol, triglycerides, urea, creatinine, T-protein, ALP, D-bili and T-bili levels. In addition, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities that had been reduced in liver, kidney, and pancreas of the treated group were restored by DSPK treatments and, therefore, the lipid peroxidation level was reduced in the liver, kidney and pancreas tissue compared to the control group. Additionally, the histological structure in these organs was restored after treatment with date seeds powder.

Keywords: antioxidant, alloxan, DSPK, oxidative stress, Phoenix dactylifera.

Resumo

O estudo foi conduzido para avaliar a atividade antioxidante e avaliar os efeitos protetores das sementes de tâmara em pó kentichi contra danos induzidos por aloxana no fígado, rim e pâncreas em ratos diabéticos. Grupo 1 (Controle): grupo controle, que não recebeu nenhum tratamento, Grupo 2 (Diab): aloxana foi injetada por via intraperitoneal (120 mg/kg de peso corporal) por dois dias, Grupo 3 (DSPK): tratado apenas com pó de sementes de tâmara adicionado na dieta (300 g por kg) por 6 semanas, Grupo 4 (DSPK+Diab): ratos aloxano-diabéticos tratados com pó de sementes de tâmaras (300 g/kg). As estimativas de parâmetros bioquímicos no sangue foram determinadas. As atividades de TBARS, SOD, CAT e GPx foram determinadas. O estudo histopatológico foi feito por meio da imersão de pedaços de ambos os órgãos em solução fixadora seguida de coloração com parafina hematoxilina-eosina. Além disso, as atividades antioxidantes de DSPK foram avaliadas pela atividade de sequestro do radical DPPH, poder redutor e sequestro do radical ABTS. Os resultados revelaram que as sementes de tâmara diminuíram significativamente os níveis séricos de glicose, colesterol, triglicerídeos, ureia, creatinina, proteína T, ALP, D-bili e T-bili. Além disso, as atividades de superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx) que haviam sido reduzidas no fígado, rim e pâncreas do grupo tratado foram restauradas pelos tratamentos com DSPK e, portanto, o nível de peroxidação lipídica foi reduzido no tecido hepático, renal e pancreático em comparação com o grupo controle. Além disso, a estrutura histológica nesses órgãos foi restaurada após tratamento com pó de sementes de tâmara.

Palavras-chave: antioxidante, aloxana, DSPK, estresse oxidativo, *Phoenix dactylifera*.

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1. Introduction

Diabetes *mellitus* is a health problem all over the world. The term "diabetes" refers to a group of heterogeneous metabolic diseases characterized by a state of chronic hyperglycemia associated to the development of microvascular complications and an increased cardiovascular risk, with varying degrees and through mechanisms that remain poorly understood, with (Monnier and Halimi, 2019). The most common forms of diabetes are type 1 and 2. Type 1 diabetes, occuring in 10% of total cases, is an autoimmune disease, while type 2 diabetes (about 90%) is oftenly associated to obesity (Ghalandari et al., 2015). Epidemiological studies revealed that, in 2019, 463 million people are suffering from diabetes and by 2045 there will be about 700 million diabetic adult (10.9% of the adult population), around the World (Saeedi et al., 2019). In general, this chronic disease occurs when the pancreas does not produce enough insulin or when the body is not able to use the insulin it produces effectively. This result in an increased concentration of glucose in the blood, this is called hyperglycemia (Skyler et al., 2017). Polydipsia, polyuria, polyphagia, and weight loss are the most common symptoms of diabetes (Kipasika et al., 2020). They develop as a consequence of functional disturbancies of major organs involved in the metabolic turnover, mainly the liver and kidney (Thomson et al., 2007).

Numerous studies suggest that diabetes is accompanied by oxidative stress that disrupts insulin secretion, promot insulin resistance and favors diabetes' pathological progression and the development of many other associated diseases (Maritim et al., 2003).

In normal conditions, the oxidative substances play a key role in many physiological activity, and are under tight regulation by antioxidative enzymes such as the catalase, superoxide dismutase and glutathione peroxidase. But, when the equilibrium of the oxidant – antioxidant system is distorted, the excessive production of oxygen radicals leads to damages in cell's structural and functional molecules such as DNA, membrane lipids and enzymes. These damages give rise to cytotoxicity, genotoxicity, and even carcinogenesis when damaged (mutated) cells proliferate (Ames et al., 1993). The main anti-diabetic agents are focusing on the induction of the endogenous insulin secretion, enhancement of the glucose uptake through specific transporters at the cell membrane, reducing the absorption of carbohydrates by the gut after food intake, as well as restraining oligo and disaccharides degradation (Chiang et al., 2014). Recently, great consensus is brought for the importance of the supplementation of diet in anti-oxidative natural products in ameliorating the therapy of diabetes and prevention of its complications (Baudin, 2020).

Several medicinal plants to treat diabetes have been used since the ancient time in many countries such as Morocco and Algeria. Among which the date palm (*Phoenix dactylifera* L.) did mainly serve for food. It is a species that belongs to the Arecaceae family (Besbes et al., 2009) and intensively grown in arid regions.

Date palm by-products (trunk, leaves, pedicels) are exploited in many domains (Djerbi, 1994). In particular, seeds of different varieties of dates that constitute between 10% and 15% of the date fruit's weight variety (Ghnimi et al., 2017) are used as feed for livestock (cattle, sheep, camels, and poultry) (Al-Farsi et al., 2007). The chemical composition of *P. dactylifera* pits has been intensively studied by several authors who revealed the presence of a large number of phenolic compounds and flavonoids which are responsible for free radical scavenging and antioxidant activities (Habib et al., 2014). Their richness in bioactive compounds suggests that they could be used for pharmaceutical researches (Baliga et al., 2011) and food industries (Sirisena et al., 2015). Numerous research works were devoted to the valuation of date seeds in different forms: activated carbon (Alhamed, 2009), preparation of citric acid and proteins (Abou-Zeid et al., 1983), bread making (Almana and Mahmoud, 1994), extraction of polysaccharides (Bouanani et al., 2007), usage in traditional medicine for its antimicrobial and antiviral properties (Jassim and Naji, 2010). According to Zangiabadi and his colleagues (2011) the extract of *P. dactylifera* date seeds might prevent against neuropathy in diabetic rats (Zangiabadi et al., 2011).

This study aimed to investigate the protective effects of *P. dactylifera* seeds against alloxan-induced diabetes Thus, liver, kidney, and pancreas damages and their related blood biomarkers and oxidative stress parameters were evaluated, in addition to phytochemical characterization of the date seeds aqueous extract.

2. Materials and Methods

2.1. Chemicals

Folin-Ciocalteu, gallic acid, catechin, quercetin, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), trichloroacetic acid (TCA), tris-buffered saline (TBS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (1%), vanillin, butylatedhydroxytoluene (BHT), potassium ferricyanide ($K_3Fe(CN)_6$) (1%), ferric chloride ($FeCl_3$), sodium chloride (NaCl), thiobarbituric acid (TBA), nitroblue tetrazolium (NBT), hydrogen peroxide (H_2O_2), catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were purchased from Sigma-Aldrich Chemicals Co. (St Louis, Missouri, USA) and Merck (Nottingham, UK). Alloxan monohydrate (Sigma USA).

2.2. Preparation of seeds extract

Mature seeds of *Phoenix dactylifera* variety 'Kentichi' (DSPK) were collected in January (2020) from the region of Degache-Tozeur oasis (southern west of Tunisia). The variety was identified by botanist Dr. Issam Saidi from the Faculty of Sciences of Gafsa (FSG), Tunisia. Dry date seeds were finely powdered and subjected to a 24- hours maceration in distilled water (1:10 weight:volume) with continual stirring at room temperature, extracted by maceration. The obtained solution was filtered, centrifugated at 3000 tr/15 min, and the supernatant was thouwed and conserved at -20 °C until further analysis.

2.3. Phytochemical screening

The phytochemical tests are qualitative tests used to determine some secondary metabolites contained in a

plant organ. Among the compounds, we can cite phenol, flavonoids and tannins.

2.3.1. Detection of polyphenols

Firstly, polyphenols in the date seeds' extract were detected by mixing 1 mL of the sample with a drop of 2% alcoholic ferric chloride ($FeCl_3$) solution. Ferric chloride interacts with polyphenols to give a dark blue or green color complex (Bidie et al., 2011).

2.3.2. Detection of flavonoids

Cribling flavonoids were carried following the method described by Bekro et al. (2007). To do so, a sample of 2 mL of the extract was mixed in 5 mL of hydrochloric alcohol (4 mL EtOH + 1 mL concentrated HCl). By adding 2 to 3 drops of magnesium a pink-orange or purplish color appears, after heating, which indicates the presence of flavonoids.

2.3.3. Detection of tannins

The identification of tannins in the extract was performed by the cribling method described by Rosine and Momo (2009). Briefly, 200 µL of 1% $FeCl_3$were added to 1 mL of the extract to produce a complex of greenish or blue-black color that indicated the presence of tannins (Rosine and Momo, 2009).

2.4. Total phenolic content

The quantification of total phenolic compounds was carried out using the Folin-Ciocalteu method described by Singleton and Rossi (1965). Breifly, 50 µL of aqueous extract was mixed with 400 µLFolin-Ciocalteu reagent. The mixture was placed for 5 min in the dark and then 500 µL of 75%sodium carbonate was added. After 1 hour of incubation in darkness, the absorbance of the solution was read at 725 nm. The total phenolic content was determined using a standard curve plotted using the gallic acid and expressed as µg of gallic acid equivalent per mL extract (µg GA/mg).

2.5. Total flavonoid content

The quantitative analysis of total flavonoids was spectrophotometrically realized according to the method described in Zhishen et al. (1999). Briefly, to 200 µL of the DSPK, were mixed with in 1 mL of distilled water with 75 µL of $NaNO_2$ (5%). Thereafter 75 µL of $AlCl_3$ (10%), and a solution of 1 mL of NaOH (1N) 8% mixed with 100 µL of distilled water, were successively added with 6 minutes- intervals of incubation between each other. The later solution was kept for 15 min at room temperature before measuring its absorbance at 510 nm. Total flavonoid content was expressed as µg of quercetin equivalent per mL extract (µg QE/mg) (Zhishen et al., 1999).

2.6. Condensed tannins contents

To estimate the total tanins' content, 20 µL of the DSPK were mixed with 600 µL of methanol vanillin solution (4%), with stirring. After 15 min, 300 µL of concentrated HCl were added and the absorbance of resultant solution was determined at 500 nm. The total tanins' concentration was given by plotting results against a standard curve using catechin as probe, and were expressed as µg of catechin equivalent per mL extract (µg CE/mg) (Makkar and Becker, 1993).

2.7. Antioxidant activity

2.7.1. Determination of the anti-radical activity against the DPPH radical

The DPPH radical scavenging activity of DSPK was evaluated using the method described by Benhammou et al. (2007). Firstly, various concentrations ranging from 50 µg/mL to 500 µg/mL were prepared by dilution of the sample in distilled water. Thereafter, 1 mL of each dilution was added to 1 mL of a 0.1 mM DPPH in methanol. The mixture was vigorously shaken and kept at 37 °C for 30 min. The absorbance of the final solution was then measured at 517 nm. The DPPH radical scavenging activity (%) was calculated by the following equation (Equation 1):

$$\%\,Iinhibition = \left[DO\,Control - DO\,sample \right] / DO\,Control \times 100 \quad (1)$$

2.7.2. Determination of the reduction power (FRAP)

The reducing power assay was determined according to the method of Chu et al. (2000). Briefly, a volume of 125 µL from various concentrations of the sample (100-500 mg/µL) was mixed with 625 µL of potassium phosphate buffer (0.1 M, pH 6.6) and 625 µL of $K_3Fe(CN)_6$ (1%); and finally 625 µL of 10% TCA were added after incubation at 50 °C for 20 minutes. The mixture was then centrifuged at 300 g for 10 min. At the end of the assay, 125 µL of 0.1% ferric chloride ($FeCl_3$) was mixed with 625 µL distilled water. After incubation for 30 min at 28 °C, the absorbance was measured at 700 nm. BHT was used as a standard synthetic positive control.

2.7.3. ABTS free radical scavenging assay

To measure the ABTS radical's neutralizing ability, 7mM ABTS radical was prepared in 2.15 mM potassium persulfate. The mixture was kept in the dark at room temperature for 16 h, and then diluted with ethanol to reach an absorbance equal to 0.70 ± 0.2 at 734 nm. To determine the ABTS radical scavenging ability of the extract, 2 mL of diluted ABTS radical were mixed with 20 µL of sample, positive or negative control (solvent). After 6 min, the absorbance was measured at 734 nm, and the ABTS scavenging activity was calculated using the following formula (Equation 2) (Re et al., 1999).

$$ABTS\,radical\,scavenging\,activity\,(\%) = \left[\left(A0 - A1 \right) / A0 \right) x100] \quad (2)$$

Where:
A_0: absorbance for control
A_1: absorbance for sample

2.8. Mineral element analysis

The determination of minerals (Cadmium, Zinc, Manganese, Copper and Chromium) contained in DSPK was performed using atomic absorption spectrometer (Hitachi Z-6100, Japan). The DSPK mineral concentrations were quantified from calibration curves of the respective standard elements.

2.9. Fourier transform infrared spectral analysis (FT-IR)

To detect chemical groups contained in Date seeds' extract we used FT-IR spectroscopy (Shimadzu, FT-IR-8400 S spectrophotometer equipped with IR solution version 1.10). The FT-IR spectra covered the infrared region 400-4000 cm^{-1}. The transmission spectra of the sample were ground by using the KBr pellets for FT-IR measurement.

2.10. Antibacterial activity

The antibacterial effects of the DSPK were verified against Gram-positive (*Staphylococcus aureus)* and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*) bacteria. The bacteria were identified and obtained from the Hospital of Metlaoui of Gafsa. The species affiliation of the examined strain was confirmed using the API-20E test kit (BioMérieux, Warsaw, Poland). Bacteria strains were maintained on Mueller-Hinton agar slopes (Oxoid) at 4 °C. According to the method of Güven et al. (2006) in triplicate.

To study the antibacterial activity of the DSPK, the disk diffusion method was used (Rhayour et al., 2003). Briefly, after 24 h of incubation at 37 °C, 4 or 5 well-isolated bacterial colonies are suspended in physiological water at 0.9% NaCl, in order to obtain a suspension containing 10^8 CFU/mL (a turbidity equivalent to 0.5 McFarland). The extracts were dissolved in DMSO to obtain a final concentrations of 100 mg/mL. Sterilized Whatman paper discs, of 6 mm in diameter, were impregnated with 20 µL of DSPK and placed on the surface of Petri dishes containing agar-agar milieu inoculated with bacteria. Discs containing 10 µg of gentamicin) were also used and served as a reference antibiotic. After pre-diffusion for one hour at a temperature of 20 °C, the Petri- dishes were incubated at 37 °C for 24 hours. The effect of the antimicrobial product on the target is assessed by measuring the diameter of the inhibition zone (Gulluce et al., 2007).

2.11. Animals

24 Wistar rats aged between 3 to 4 months and weighing 150 ± 20 g wer breeded at then animal house of the Faculty of Sciences of Gafsa, Tunisia. Rats were allowed to acclimate to thousing condition (temperature of 24 ± 2 °C, air humidity of 40% and a light-dark cycle of 12 hours) for two weeks. They received standard food pellets and tap water. They were caged by group of six rats, each. Our study design follows the guidelines of the Directive 2010/63/EU of the European parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes and was approved by Gafsa University Animal Ethics Committee (G/A/SV/2016/001).

2.12. Experimental induction of diabetes mellitus

Rats were injected with a freshly prepared solution of alloxan monohydrate in saline (300 mM NaCl) at a dose of 120 mg/kg body weight as proposed by Hfaiedh et al. (2013). Since alloxan injection can provoke fatal hypoglycemia as a result of reactive massive release of insulin, rats were also given orally 5-10 mL of a 20% glucose solution after 6 h. Rats were then kept for the next 24 h on a 5% glucose solution as a beverage to prevent severe hypoglycemia.

2.13. Acute toxicity study

The rats were fasted for 12 h and divided into 8 groups of five rats each. The DSPE extract was administrated orally (Hodge and Sterner Scale) with increasing doses of 1000 mg/kg (1000-12,000 mg/kg). After administration of this extract, the animals were observed continuously for 24 h (with special attention given during the first 2 h). Changes in the normal activity of rats due to acute toxicity were monitored. The lethal dose that killed 50% of the mice was estimated after 24 h, applying the method of Miller and Tainter (1944).

2.14. Experimental design

Animals were randomly distributed into 4 groups of 6 rats each. All rats were individually followed-up for at least 30 minutes per day, in order to check for any signs of intoxication during the overall period of experimentation.

The first group (control) served as negative control and comprises non-diabetic normal rats nourished with standard food pellets.

The group 2 (Diab) consisted of diabetic rats (receiving alloxan injection) that have been feeded standard pellets. Rats from the third group (DSPK) are non-diabetic and received food supplemented with 300 g/kg of DSPK. Rats of the last group were diabetic and have been nourished by pellets supplemented with date seeds' powder at the same concentration (Diab + DSPK).

2.15. Serum analysis

After six weeks, rats were euthanized by decapitation and arterio-venous blood was collected and centrifuged at 3000 rpm for 10 minutes. Plasma samples were then aspirated and conserved in conical test tubes at 80 °C. Organs were also harvested for oxidative stress parameters and histological studies (Aljehany, 2021).

2.16. Biochemical assays

The blood concentration of glucose, total cholesterol, triglycerides, urea, creatinine, total protein, alkaline phosphatase, D-bilirubin, and T-bilirubin were determined using automated apparatus according to the manifacturer's instruction (Spinreact). Tissues content in protein were determined in their lysates by the Lowry's method (Lowry et al., 1951) using bovine serum albumin (BSA) as standard.

2.17. Preparation of liver, kidney and pancreas extracts

1 g of the organs, were cut into small pieces, immersed in 2 mL of TBS (Tris-buffered-saline: pH=7.4) using an Ultra-Turrax grinder, and centrifugated (15 min at 4 °C). Supernatants were stored at -80 °C until use.

2.18. Oxidative stress tests

2.18.1. Determination of the lipid peroxidation level

The lipid peroxidation level was measured as thiobarbituric acid reactive substances (TBARS) according

to Yagi (1976). To perform this assay, 125 µL of supernatant (S1) was mixed with 175 µL of 20% trichloroacetic acid containing 1% butyl-hydroxytoluene and centrifuged (1000 g, 10 min, 4 °C). Then, 200 mL of supernatant (S2) was mixed with 40 µL of HCl (0.6 M) and 160 µL of thiobarbituric acid (0.72 mM), and the mixture was heated at 80 °C for 10 min. The absorbance was measured at 530 nm. The amount of TBARS was calculated using an extinction coefficient of 156 mM^{-1} $cm^{-1}$and expressed as nmol/mg protein.

2.18.2. The total superoxide-dismutase (SOD) activity

SOD levels was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) (Misra and Fridovich, 1972). One unit of SOD represents the amount inhibiting the photoreduction of NBT by 50%. The activity was expressed as units/mg protein, at 25 °C.

2.18.3. Catalase (CAT) activity

The CAT was measured according to Aebi (1984). The reaction mixture (1 mL) contained 100 mM phosphate buffer (pH = 7), 100 mM H_2O_2, and 20 µL (about 1-1.5 mg of protein) of the kidney. H_2O_2 decomposition was tracked at 25 °C by measuring the decrease in absorbance at 240 nm for 1 min. Enzyme activity was calculated using an extinction coefficient of 0.043 mM^{-1} cm^{-1} and expressed in international units (I.U.), in mmols of H_2O_2 destroyed/min/mg protein.

2.18.4. Glutathione-peroxidase (GPx) activity

The GPx was assayed according to the method of Flohé and Günzler (1984). One unit of GPx was defined as oxidation by H_2O_2 of 1 µL of reduced glutathione peroxide per min at a pH of 7 and a temperature of 25 °C.

2.19. Histological study

After harvesting, organs were gently separated from their adjacent tissues and weighed. Thereafter, fragments of liver, kidneys, and pancreas were quickly excised and fixed into 10% of formaldehyde, at least for 48 hours. Tissues were then enrolled into paraffin and slides of 5 µm thickness were stained with hematoxylin-eosin. The examination of slides was done under light microscope.

2.20. Statistical analysis

All in vitro tests were performed in triplicate and their results are presented as means±standard deviation (SD). Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by the Fisher's tets, using SPSS program for Windows.17 (IBM corporation). The significance was considered at $p<0.05$. The LD_{50} values were calculated by probit analysis with a reliability interval of 95%.

3. Results

3.1. Yield of extraction

The aqueous extraction obtained by maceration from DSPK gave a significant yield of 14%.

3.2. Phytochemical studies of DSPK

Qualitative phytochemical analysis of DSPK demonstrated the presence of phenol, flavonoids and tannins. The contents of total phenolic compounds, revealed a significant amount of polyphenols (209.3 ± 0.5 µg GAE/mg), flavonoids, and condensed tannins (65.6 ± 0.20 µg QE/mg and 16.61 ± 3.66 µg TAE/mg, respectively) (Table 1).

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

Figure 1 illustrate the antiradical activity of DSPK presented by IC_{50} value which is defined as the concentration of the antioxidant required to scavenge 50% of DPPH and calculated by a graph plotting. The radical scavenging activity of DSPK and ascorbic acid (AA) on DPPH radicals increases as concentrations increase. The IC_{50} values determined from the graph show that the radical-scavenging activity of DSPK (IC_{50}= 450 ± 1.54 µg/mL) appeared lower than that of ascorbic acid used as a positive control (IC_{50}= 100 ± 0.12 µg/mL).

3.3.2. Ferric reducing antioxidant power

As shown in Figure 2, date seeds can reduce Fe^{3+} to Fe^{2+}, at different concentration ranges. The reducing power of DSPK and its concentration are concentration-dependent. It was found to be 0.9 ± 0.068 absorbance units at 500 µg/mL. This activity appeared significantly ($p < 0.05$) lower compared with that of the positive control (BHT), which was 1.02 ± 0.26 absorbance units at 500 mg/mL.

Table 1. Phytochemical content of DSPK.

	Contents	
[1]Polyphenols	209.3 ± 0.5 µg GAE/mg	+++
[2]Flavonoids	65.6 ± 0.20 µg QE/mg	++
[3]Condensed tannins	16.61 ± 3.66 µg TAE/mg	++

[1]Total phenolic content as the gallic acid equivalent. [2]Total flavonoid content as the quercetin equivalent. [3]Condensed tannin as the tannic acid equivalent. Results are expressed as mean of three experiments ± SD. The number of determinations was n = 3. ++Presence; +++High presence.

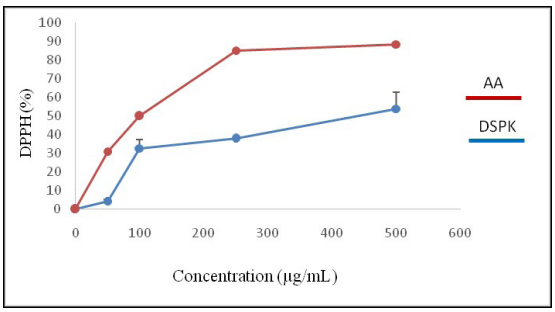

Figure 1. The antiradical activity of DSPK against the radical DPPH. Values are represented as mean standard deviaton (n = 3).

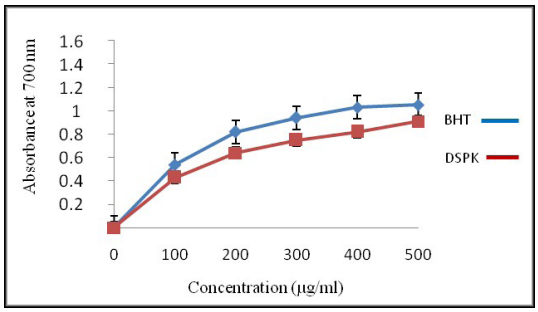

Figure 2. The reducing power of DSPK and BHT by the FRAP assay. Values are expressed as mean standard deviation (n = 3).

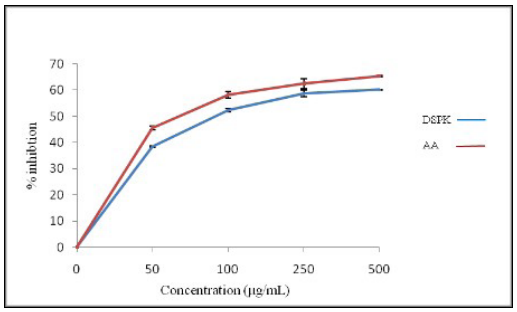

Figure 3. The antioxidant activity of DSPK against the ABTS. Values are represented as mean standard deviation (n = 3).

3.3.3. ABTS free radical scavenging assay

The antioxidant activity to scavenge ABTS radical was determined in equivalent to ascorbic acid (AA). As shown in Figure 3, the ability of the tested samples to scavenge the radicals of (DSPK)and (AA) on ABTS radicals increases as concentrations increase. The IC_{50} values determined from the graph show that the radical-scavenging activity of DSPK (IC_{50} = 94 ± 0.4 µg/mL) appeared lower than that of ascorbic acid used as a positive control (IC_{50} = 70 ± 0.2 µg/mL).

3.4. Mineral composition of DSPK

The atomic absorption spectrometry showed that it contains great amount of minerals. In particular, chromium and zinc were the major represented ones (51 ± 0.11 ppm and 16 ± 0.45 ppm, respectively) copper and cadmium was of the lowest depicted values in DSPK (Table 2).

3.5. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis shown in Figure 4. The spectrum reveals two bands within vibration frequency ranging from 3450 to 3500 $cm^{-1}$and 1620 to 1635 cm^{-1} that are attributed to two chemical functional groups of phenolic compounds: the sailing hydroxyl (-OH) radical and double carbon bounds (C=C) of aromatic chemicals.

3.6. Antibacterial activity

In the present study, the antibacterial activities of DSPK were screened against 4 bacterial strains. Our findings revealed that the diameter of the inhibitory zone ranged between 8 and 15 mm with maximal growth inhibition against *Pseudomonas aeruginosa* (15 ± 0.052 mm) (Table 3). The effect of DSPK was about the half of which of gentamicin, in the overall tested strains.

3.7. Acute toxicity

Lethal dose (LD_{50}) values were estimated using Finney's probit analysis program. The acute toxicity study showed no mortality and any signs of toxicity at all dose of DSPK extract administered up to 5000 mg/kg, indicating that the *Phoenix dactylifera* extract is safe even at higher doses. The LD of the extract in rats was recorded at 11,245 mg/kg and was considered to be non-toxic plant according to Hodge and Sterner Scale.

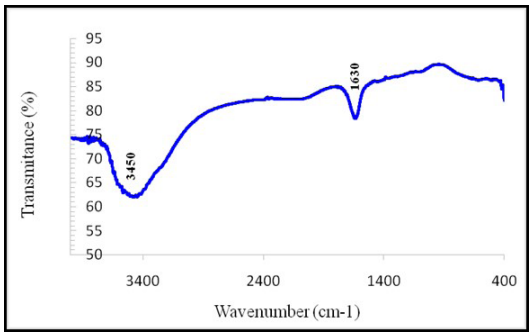

Figure 4. Fourier transform infrared (FT-IR) spectrum of DSPK.

Table 2. Mineral composition of DSPK.

Mineral elements	DSPK (ppm)
Chromium (Cr)	51 ± 0.11
Zinc (Zn)	16 ± 0.45
Manganese (Mn)	11 ± 1.01
Copper (Cu)	2 ± 0.62
Cadmium (Cd)	2 ± 0.12

Table 3. Antibacterial activity of DSPK and antibiotic against for bacterial strains.

Inhibition zone (mm)		
Bacterial strains	**DSPK**	**Gentamicin**
Staphylococcus aureus	8 ± 0.20	20 ± 0.035
Pseudomonas aeruginosa	15 ± 0.052	30 ± 0.03
Escherichia coli	9 ± 0.034	25 ± 0.04
Klebsiella pneumoniae	13 ± 0.11	30 ± 0.05

3.8. Weight of body, liver, kidney, and pancreas

The measurement of the whole body weight showed significant differences between the experimented groups of

rats, at the end of the experiment (Table 4). In comparison to the control group (183.33 ± 3.4 g), only diabetic rats presented a significant ($p < 0.05$) decrease in their body weight (152.5 ± 2.33 g). This lowered growth was re-established by the intake of DSPK supplemented diet (Diab + DSPK). However, the relative size of the studied organs did not present significant changes between the experimented groups.

3.9. Food and water intake

Accordingly, Table 5 clearly shows the excessive augmentation in water consumption ($p < 0.01$) and diminishing in food intake ($p < 0.05$) by diabetic rats (8.3 ± 2.45 ml/day/rat and 9 ± 3.43 g/day/rat, respectively), in comparison to control group (23.87 ± 1.5 ml/day/rat and 16.5 ± 2.63g/day/rat, respectively). A full recovery of both polydipsia and polyphagia was observed in DSPK-treated diabetic rats.

3.10. Biochemical analyses

The administration of alloxan alone to rats resulted in a significant increase of glycemia (11.28 ± 2.25 g/L) when compared to the control group (7.71 ± 0.58 g/L) (Figure 5). However, the intake of DSPK- supplemented diet by diabetic rats (Diab+DSPK) induced a significant diminishing in blood's glucose concentration (8.96 ± 1.06) but remains higher than in normal values in control group ($p < 0.05$).

Similar changes were also observed in all the studied diagnostic parameters of liver and kidney functions for example, the conjugated bilirubin (1.72 ± 0.31 µmol/L) and alkaline phosphatase (208.33 ± 8.62 U/L) values were, respectively, 2 and 1.5 folded in Diab group in comparison to control (0.63 ± 0.12 µmol/L and 140 ± 19 U/L, respectively), while they returned to normal range within treatment of diabetes by DSPK (1.01 ± 0.27 µmol/L and 141.33 ± 39.80 U/L, respectively for D-Bil and ALP). Impressively, the lipidic profile of diabetes was also full re-established by the intake of the palm date product (Table 6). The renal function was characterized by a significant levelling up of creatinine, blood nitrogen urea and total proteins' blood concentrations in alloxan-induced diabetes. The DSPK alone, did not influence normal values of these parameters, but it significantly resulted in their depletion when co-administred with alloxan (Table 6).

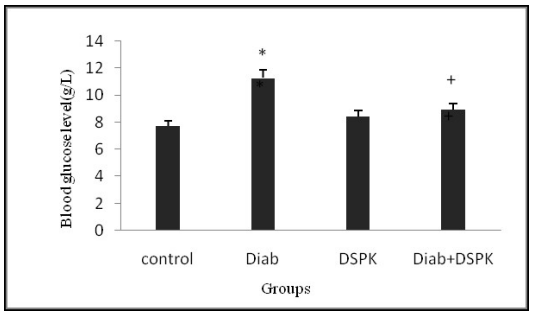

Figure 5. Effect of DSPK on serum glucose levels in control: normal rats; Diab: alloxan diabetic rats; DSPK: rats treated with DSPK; DSPK+Diab: treated with DSPK then with alloxan. Values are the mean of 6 measurements ± SD. *Significant difference as compared to control rats ($p < 0.05$); +($p < 0.05$) Significant difference when compared to Diabetic group.

Table 4. General characteristics of body, liver, kidney, and pancreas weight in the experimental group.

Parameters (g)	Control	Diab	DSPk	DSPK+Diab
Initial body weight	126.66 ± 3.82	127 ± 2.02	124 ± 2.03	127.5 ± 1.51
Final body weight	183.33 ± 3.4	152.5 ± 2.33*	176 ± 1.08	179 ± 2.07+
Weight of the liver	4.26 ± 1.15	4.1 ± 0.29	3.33 ± 0.9	3.8 ± 0.57
Weight of the kidney	1.04 ± 0.12	1.2 ± 0.14	1 ± 0.1	1.1 ± 0.095
Weight of the pancreas	0.75 ± 0.20	0.6 ± 0.05	0.73 ± 0.20	0.63 ± 0.17

Values are the mean of 6 measurements ± SD. *Significant difference as compared to control rats ($p < 0.05$). +Significant difference when compared to Diabetic group ($p \leq 0.05$).

Table 5. General characteristics of food and water intake in the experimental group.

Groups	Water intake before injection Alloxan (mL/day/rat)	Water intake after injection Alloxan (mL/day/rat)	Foods intake before injection Alloxan (g/day/rat)	Foods intake after injection Alloxan (g/day/rat)
Control	22.5 ± 2.81	23.87 ± 1.5	15 ± 1.02	16.5 ± 2.63
Diab	25 ± 1.25	83.3 ± 2.45**	14 ± 4.55	9 ± 3.43*
DSPK	21.83 ± 1.05	22 ± 2.42	14 ± 1.71	14.1 ± 2.9
DSPK + Diab	20.5 ± 1.40	41 ± 4.5+	11.5 ± 1.43	13.66 ± 2.61+

Values are the mean of 6 measurements ± SD. *Significant difference as compared to control rats ($p < 0.05$). **Significant difference as compared to control rats ($p < 0.05$). +Significant difference when compared to diabetic group ($p < 0.05$).

3.11. Anti-oxidant and tissues' protective effect of DSPK

Table 7 summarizes the measured oxidative parameters in various organs of the experimented groups. It reveals a significant increase in the malondialdehyde (TBARS) that forms as a consequence of alloxan- induced diabtes (Diab) (0.077 ± 2.14 UI/mg of proteins) in comparison to all other group of rats such as the control (0.022 ± 0.25 UI/mg of proteins) and (Diab+DSPK)- ones (0.033 ± 1.33 UI/mg of proteins), in hepatic tissues. The most important change was noted in pancreatic tissues where TBARS value reached in Diab 4.5-folds which of the control. A such effect was accompanied by a respective significant inhibition of the anti-oxidative enzymes' activities in both liver, pancreas and kidney by 7% to 50% in Diab group when compared to other experimented rats. These finding bring clear proof for the *in vivo* anti-oxidant activity of our natural product. Furthermore, diabetes induced decrease in CAT, SOD and GPx activities were observed in diabetic rat liver, kidney and pancreas compared with the control group. However, the administration of DSPK to diabetic rats reverted the above antioxidant activities towards normal levels (Table 7).

Table 6. Effect of administration of DSPK on liver profile indices: total cholesterol (T-Ch), triglycerides (TG), alkaline phosphatase (ALP), total bilirubine (T-Bili) and direct bilirubine (D-Bili) and kidney parameters as creatinine, urea and total protein of control and experimental groups of rats.

Liver	Control	Diab	DSPK	DSPK + Diab
T-Ch (mmol/L)	1.49 ± 0.13	1.71 ± 0.10*	1.45 ± 0.05	1.52 ± 0.04+
TG (mmol/L)	1.36 ± 0.05	1.63 ± 0.09*	1.31 ± 0.06	1.35 ± 0.11+
ALP (U/L)	140 ± 19	208.33 ± 8.62**	117.66 ± 19.13	141.33 ±39.80++
D-Bili (µmol/L)	0.63 ± 0.12	1.72 ± 0.31+	0.24 ± 0.14	1.01 ± 0.27++
T-Bili (µmol/L)	14.37 ± 2.12	19.83 ± 2.73+	14.65 ± 0.21	14.12 ± 1.35+
Kidney				
Creatinine (µmol/L)	42.53 ± 4.01	49.76 ± 1.92**	42.73 ± 2.85	40.76 ± 5.56++
Urea (mmol/L)	4.41 ± 0.14	5.89 ± 0.30+	4.48 ± 0.30	4.74 ± 0.66+
T-Protein (g/L)	70.23 ± 2.41	75.06 ± 4.24+	63.16 ± 2.70	63.93 ± 1.79++

Values are the mean of 6 measurements ± SD. *Significant difference as compared to control rats (p <0.05). **Significant difference as compared to control rats (p <0.05). ++Significant difference as compared to diabetic rats (p <0.05). +Significant difference when compared to diabetic group (p <0.05).

Table 7. Effect of DSPK administration on CAT, SOD, GPx and TBARS levels in liver, kidney and pancreas incontrol: normal rats; Diab: alloxan diabetic rats; DSPK: rats treated with DSPK; DSPK+Diab: treated with DSPK then with alloxan.

Groups / Liver	TBARS	CAT	SOD	GPx
Control	0.022 ± 0.25	11.53 ± 0.98	84.66 ± 2.52	139.79 ± 2.88
Diab	0.077 ± 2.14*	7.35 ± 0.13*	79.46 ± 0.11*	79.5 ± 0.54**
DSPK	0.021 ± 0.32	11.11 ± 3.78	135 ± 0.64	142 ± 0.97
DSPK+Diab	0.033 ± 1.33+	8.33 ± 0.22+	113.51 ± 2.78++	104.32 ± 1.87++
Kidney				
Control	0.046 ± 0.22	8.88 ± 0.22	139.4 ± 0.44	87.36 ± 0.21
Diab	0.054 ± 4.02*	4.54 ± 0.87**	93.2 ± 0.62**	39.07 ± 0.75**
DSPK	0.048 ± 2.81	12 ± 1.87	203.42 ± 0.01	76.3 ± 1.88
DSPK+Diab	0.05 ± 1.54+	9.3 ± 0.54++	96.2 ± 1.92+	45.32 ± 2.31+
Pancreas				
Control	0.028 ± 2.55	50 ± 0.02	182.6 ± 1.30	153.93 ± 0.65
Diab	0.129 ± 0.21*	35.72 ± 0.44*	109.94 ± 1.64**	77.5 ± 0.04**
DSPK	0.038 ± 1.54	53 ± 2.31	183 ± 2.50	253.65 ± 4.54
DSPK+Diab	0.039 ± 2.87	48.14 ± 0.87+	122 ± 1.33++	172.66 ± 5.01++

Values are the mean of 6 measurements ± SD. *Significant difference as compared to control rats (p <0.05). **Significant difference as compared to control rats (p <0.05). ++Significant difference as compared to diabetic rats (p <0.05). +Significant difference when compared to Diabetic group (p <0.05).

3.12. Histological study

The *in vivo* antioxidant activity goes along with the histological results (Figures 6, 7 and 8) that confirm the hepatic, renal and pancreatic toxicity induced by alloxan-induced diabetes. The liver manifests an abundant necrosis of hepatocytes that disturbs hepatic trabeculae (sinuses) and infiltrating immune cells. There was also a congestive features of the portal space, then in the kidney observed an atrophies in the glomerules and increase in size capsular space (Figure 7). It was also noted to induce degenerative islets of Langerhans in the pancreatic tissue, in Diab group. The SDPK supplementation lead to substantial resolution of these hepatic, renal and pancreatic damages (SDPK + Diab) where it did not influence the liver architecture when administered alone. The intake of date seeds powder (SDPK+Diab) did, also, restore the damages induced by alloxan-injection in kidney and pancreas moderate protective action of β-cells was observed and to restore the affected values back which revealed problem of lipid accumulation of fat cells in liver of diabetic rats as compared to control rats (Figures 6, 7 and 8).

4. Discussion

Diabetes is a metabolic disorder belonging to a group of non-communicable diseases. It remains a major public health problem because of its serious morbid consequences and its progressive nature (Kerekou et al., 2014). It generally associates to several pathological status such as obesity, and hypertension, retinal degeneration and cardiovascular diseases bias a mechanism involving the oxidative stress (Vona et al., 2019). Mainly, the treatment of this expending pathology focuses on restoring glycemia through both pharmacological and non- pharmacological strategies (Khursheed et al., 2019). Among new approaches to prevent and treat diabetes mellitus (DM), dietetic and phytotherapeutic are gaining much of interest. In such context, we evaluate the effect of diet supplementation by *P. dactilyfera* seeds against DM. Our results revealed the richness of this plant by- product in phenolic compounds that are shown to reduce the oxidative stress and to treat many diseases in traditional medicine. Accordingly, the DSPK showed relevant scavenging free- radicals potential which is mainly attributed to the hydroxyl chemical function contained in polyphenols, flavonoids and tannins (Sun et al., 2004; Sakr et al., 2015; Chiorcea-Paquim et al., 2020).

Furthermore, it inhibits several bacterial strains (Al-Daihan and Bhat, 2012), that might help ameliorating diabetes patients' cases. The intake of food supplemented by DSPK relieved DM and restored the histological damages related to diabetes. Similar findings have been reported by many authors (Mia et al., 2020; Barakat et al., 2020; Abu-Odeh and Talib, 2021). While the real mechanism

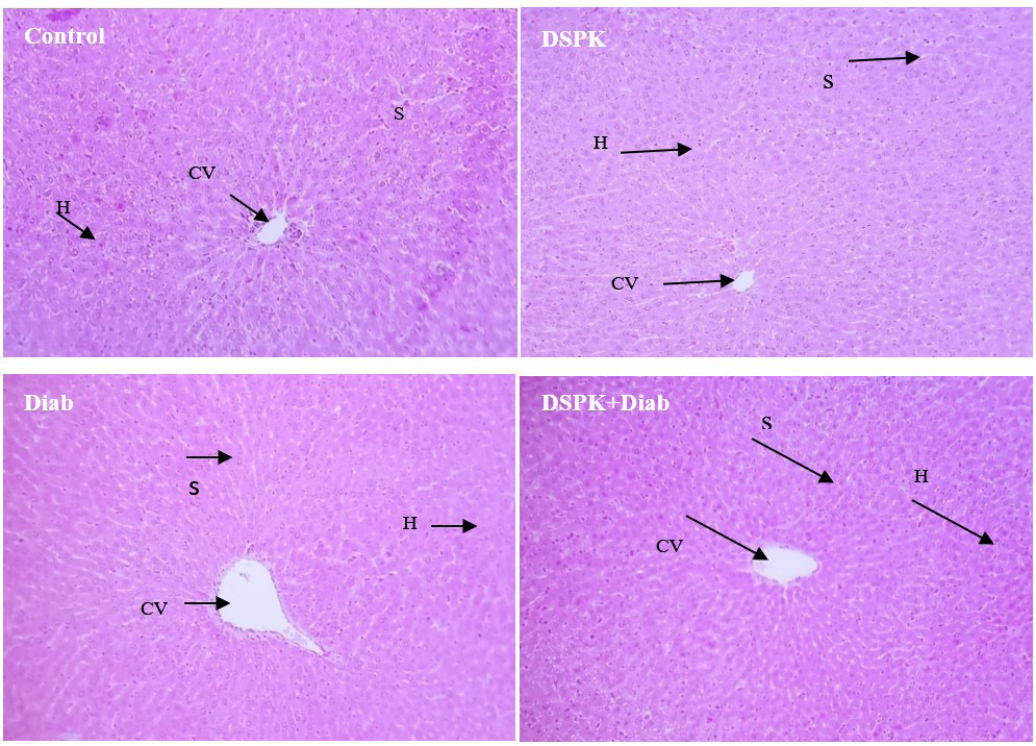

Figure 6. Microscopic observations of rat liver sections (HE × 10, Control, DSPK, Diab, and DSPK + Diab). Control: control group shows normal parenchymal architecture with normal Central vein (CV), hepatocytes (H), and blood sinusoids (S). DSPK: rats treated with date seeds powder of *P. dactylifera* L. showing normal structure similar to control; Diab: rats treated with alloxan demonstrating liver in the portal area that associated with severe congestion and dilatation of Central vein (CV) and necrosis of the surrounding hepatocytes and DSPK + Diab: rats pretreated with DSPK then treated with alloxan showing repairing of the liver structure.

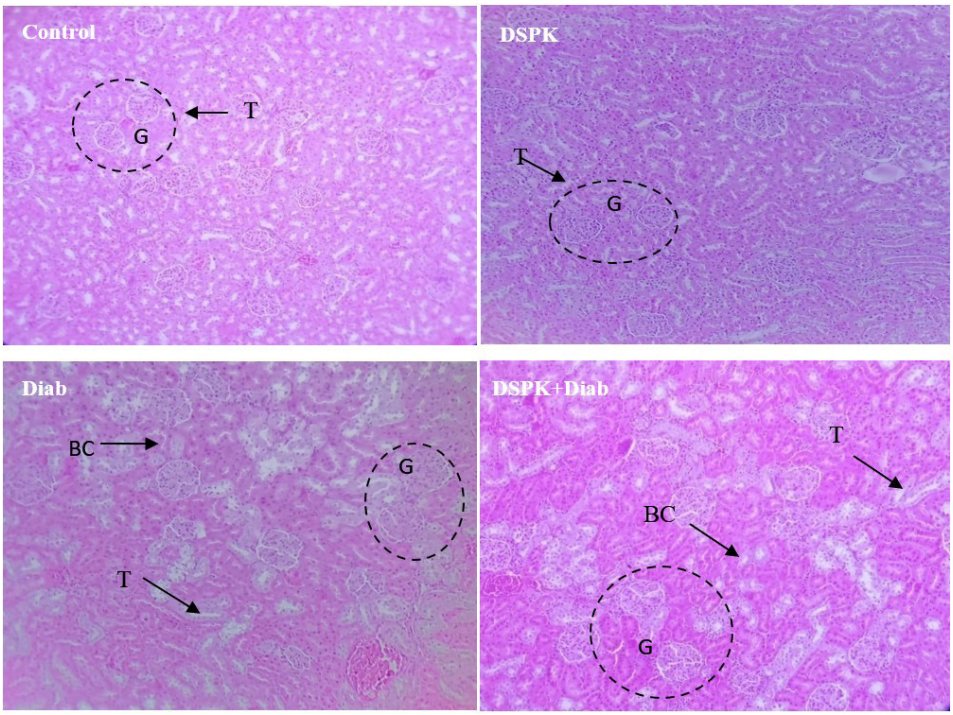

Figure 7. Microscopic observations of rate kidney (HE × 10, Control, DSPK, Diab, and DSPK + Diab). Control: control group showing normal parenchymal architecture; DSPK: rats treated with date seeds powder of *P. dactylifera*; Diab: rats treated with alloxan showing cytoplasmic vacuolization of epithelial lining renal tubules (arrow) and degeneration changes and DSPK + Diab: rats pretreated with DSPK then treated with alloxan showing repairing of the kidney structure. G: Glomerule; T: Tubule; BC: Bowman's capsule.

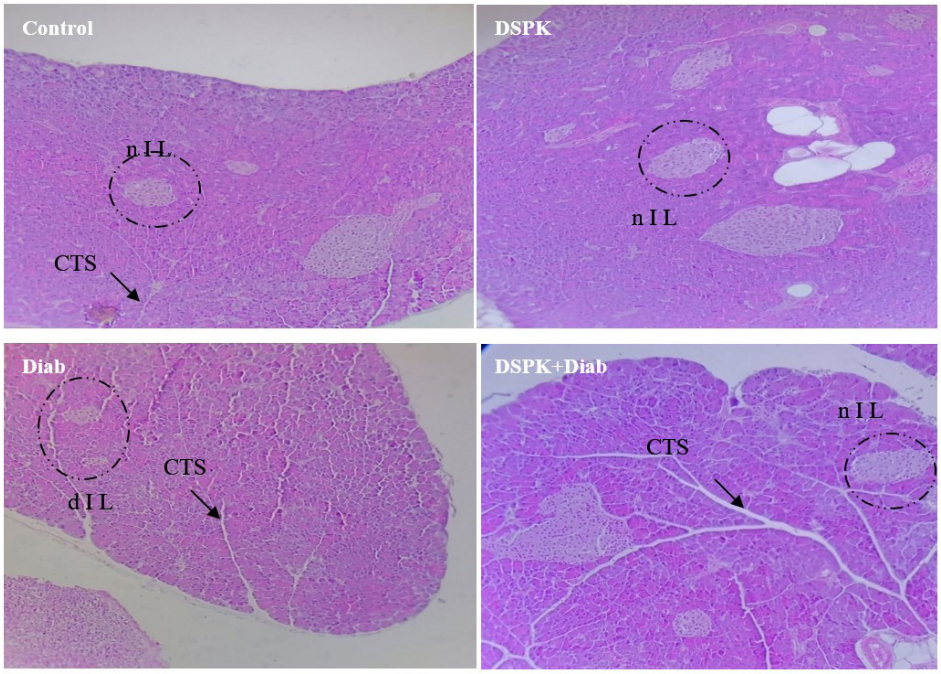

Figure 8. Microscopic observations of rate pancreas (HE × 10, Control, DSPK, Diab and DSPK + Diab). Control: control group showing normal exocrine acini and endocrine islets; DSPK: rats treated with date seeds powder of *P. dactylifera*; Diab: rats treated with alloxan showing degenerative and necrotic alterations, contracted in the islet of Langerhans, β-cells and diminished islet cell density and DSPK + Diab: rats pretreated with DSPK then treated with alloxan showing repairing of the pancreas structure; nIL: normal islets of Langerhans; dIL: degenerated islets of Langerhans; CTS: connective tissue septa.

of action of the anti-diabetic effect of date seeds is still unclear, it is suggested that its *modus operandi* involves anti-inflammatory and anti-oxidative pathways (Barakat et al., 2020; Moslemi et al., 2022).

The re-establishment of biomarkers of hepatic and renal function; found herein, supports findings reported by Salama et al. (2019) who found that DSPK intake reduces levels of glucose, T-cholesterol, triglycerides, and the main indicators of renal function in diabetic rats DSPK has been proved to act synergistically with insulin to reduce hyperglycemia and prevent secondary outcomes of diabetes mellitus such as cardiovascular events and anxiety (Bikri et al., 2021).

In effect, according to El-Fouhil and her colleagues, DSPK stimulates the secretion of insulin, that is a sought endpoint in DM pharmacological therapy (El Fouhil et al., 2013) and inhibit several metabolic enzymes regulation lipidemia and glycemia (Djaoudene et al., 2019).

The improvement of the diabetic status was also correlated to the protective effect of DSPK against hepatic, pancreatic and renal damages that results from cues of endogenous inflammatory and pro-inflammatory factors in diabets (Saryono et al., 2019).

The oxidative stress originating from the disequilibrium in the oxidant – antioxidant system, obviously, leads to tissues damages through oxidizing cellular components such as DNA, proteins and lipids (Schlorff et al., 1999). Herein, we found that the administration of date seeds powder induces the recovery of this system balance and protects both liver, kidney and pancreas tissues, in similar manner reported by other authors (Abdelaziz et al., 2015). In addition to phenolic compounds' free radicals neutralization, DSPK contains several minerals such as zinc and copper (Abdul Afiq et al., 2013; Ali-Mohamed and Khamis, 2004; Besbes et al., 2004) that form co-factors for antioxidant enzymes and stimulate their functions (Huchzermeyer et al., 2022).

Because of the profound metabolic alteration caused by diabetes mellitus type 1, there is generally loss of body weight (Daisy et al., 2012). Thus, the recovery of hepatic and pancreatic structures and functions might associate to body weight increase in diabetic rats treated by DSPK. Date pits have been used for human and livestock nutritional purposes (Al-Taher, 2008; Maqsood and Benjakul, 2010), and was considered as generally safe. Hence, it might serve as good dietetic supply of adjuvant for diabetes mellitus management.

5. Conclusion

Seeds of Date palms (*Phoenix dactylifera* L.) are rich by-products in polyphenolic components and minerals that prevent the development of diabetes mellitus and its associated diseases through a mechanism, mainly, involving its anti-oxidant properties. Since it is a commonly used by several populations for different nutritional purposes, it is considered as generally safe and can be enrolled in approaches of diabetes mellitus management, as dietetic supply or adjuvant. Further inspections of its real molecular mechanism of action regulating glycemia are being investigated to better advance its usefulness for clinical application.

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