EFFECTS OF PALM DATES (PHOENIX DACTYLIFERA L) EXTRACTS ON HEPATIC DYSFUNCTIONS IN TYPE 2 DIABETIC RAT MODEL

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ABSTRACT

Phoenix dactylifera L is one of the most useful traditional medicinal plants. Previous studies demonstrated that it has many pharmacological actions such as antibacterial, anti-inflammatory, anti-diabetic, anti-asthamic, nephroprotective and hepatoprotective activities. Moreover, previous studies demonstrated that its fruits and seeds contain many chemical substances such as anthocyanins, phenolics, sterols, carotenoids and flavonoids. So, the present study was designed to analyze its phenolics and flavonoids contents using HPLC – PDA as well as to investigate the protective properties of palm date extracts on liver functions in type 2 diabetic rats. The results of this study revealed that the extracts of palm dates contain phenolic compounds caffeic, gallic, ferulic, p-coumaric, sinapic, chlorogenic acid and falvonoid compounds Apegenin, Quercetin and luteolin glycosides in fruit extracts and vanillic acid, gallic acid, caffeic acid, p-Coumaric acid and quercitin in seed extracts. Also, both fruit and seed extracts caused significant improvement in glycaemic control and liver functions in diabetic rats. We concluded that both fruit and seed extracts have hepatoprotective effect in type 2 diabetic rats which might be due to their phenolics and flavonoids contents.

KEY WORDS: Phoenix dactylifera; phenolics; flavonoids glycoside, High Performance Liquid Chromatography (HPLC); diabetes and hepatoprotective.
INTRODUCTION
Phoenix dactylifera L. tree and its products were regarded as vegetables with health benefits and have been employed traditionally to remedy a number of pathological conditions.\[1\] Date fruits are a significant component of the diet in the majority of the Arab countries with low cost. Egypt is considered one of the major producers of dates in the middle East with 17% of the world production.\[2\] The importance of the date in human nutrition comes from its rich composition of carbohydrates (70–80%), salts and minerals, dietary fiber, vitamins, fatty acids, amino acids and protein.\[3,4\] Research proves that when dates are eaten alone or as mixed meals with yoghurts they have low glycemic indexes.\[5\] Unlike most other fruits, dates can be consumed at any of the three major stages of maturity such as khalal or besser (fresh, hard ripe, color stage), rutab (crisp to succulent or ripe stage), or tamr (soft and pliable, fully ripe stage).\[1\] The date fruit pulp is rich in phytochemicals like phenolics, sterols, carotenoids, anthocyanins, procyanidins, and flavonoids.\[6-11\] The date pits which are a waste product have been used for centuries in the Arab world to make caffeine-free drink. Recently, date pit powders are also marketed and are a source of choice to people preferring a non-caffeinated coffee with coffee-related flavor.\[12\] The fleshy tissues of dates contain 0.2-0.5% oil, while the seed contains 7.7-9.7% oil.\[13\] The phenolic acids detected in date seed were gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, m-coumaric acid and o-coumaric acid.\[12\]

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or action or both, that affects carbohydrate, lipid, protein and nucleic acid metabolism.\[14\] Liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and also synthesize glucose from non-carbohydrate sources. This key function of liver makes it vulnerable to diseases in subjects with metabolic disorders, particularly diabetes.\[15\] Previous studies reported increased activities of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GGT) (markers of liver injury) in cases of insulin resistance (16), metabolic syndrome and type 2 diabetes.\[17-19\] The onset of diabetes is accompanied by development of major biochemical and functional abnormalities in the liver, including alterations in carbohydrate, lipid, and protein metabolism, and changes in antioxidant status.\[20-23\] It has been demonstrated that, reactive oxygen species (ROS) could play a key event in the development of diabetes complications such as hepatic injury\[24, 25\] which induce rapidly induces apoptotic
cell death.\textsuperscript{[26]} Up to the best of our knowledge, there is no study investigated the effect of palm date extracts on hepatic injury in DM. So, this study was designed to investigate the effect of palm date extracts on hepatic injury secondary to type 2 DM.

MATERIAL AND METHODS

Collection and extraction of Palm date fruits and seeds

Palm date (Phoenix dactylifera L.) were collected at tamr stage in Sept 2013 from Sharkyia Governorate and kept in a refrigerator at 4 °C and identified by Botany department, Faculty of Science, Zagazig University.

Date flesh was manually separated from the pits and 100 gm of fruits crushed and cut to small pieces with a sharp knife and 100 gm of seeds were ground into a fine powder are taken in a flasks. The fine powder of each part were separately taken into flasks and immersed and extracted three times with 500 ml of n-hexane. The flask was shaken for 24 hours with 3 hours interval using a magnetic stirrer, and then filtered with Whatman filter paper. The solvent was removed under reduced pressure at 40°C using a rotary evaporator to obtain hexan crude extracts.

Defatted (using n-hexan) fruits and seeds were separately extracted three times with methanol (500 ml) at room temperature for 24 h using a magnetic stirrer, then methanolic extracts were filtered and centrifuged at 6000 radius centrifugation force (RCF) or G-force, for 30 min at 3°C and the supernatant were concentrated under reduced pressure at 40°C for 1 to 2 h using a rotary evaporator to obtain methanol crude extracts. N-hexan and methanol extracts were kept in dark glass bottles at -4°C until used. Also, the dry date palm fruits were soaked in water (1:5 w/v) at 40°C then stirred for 24 hrs at 4°C temperature. The mixture was centrifuged at 6000 RCF for 20 min to discard the precipitate and one part of supernatant was lyophilized and stored at 4°C for further analysis.\textsuperscript{[27]}

The composition of major phenolic substances was determined by HPLC - PDA analysis. The typical chromatogram recorded at 280 and 340 nm. Phenolics and flavonoids contents of extracts were tentatively identified and quantified in the investigated fruits by comparison with the retention times and UV spectra of standards that were analyzed under identical conditions.
Isolation and identification of phenolic compounds in aqueous and methanolic fruits and seeds extracts by chromatography

Two dimensional paper chromatography of the extract was applied on Whatman paper No 1, irrigated in the solvent system 6%(acetic acid –water ) (HOAC-6), followed by butanol: acetic: water (4:1:5) revealed the presence of mainly Eight phenolic compound in fruits extracts and five in seeds extracts, corresponding spots gave positive response toward fcl3 spray reagent, some of which appeared under UV light as dark purple spots which turned orange or lemon yellow or reddish orange when fumed with ammonia vapor or when sprayed with Naturstuff spray reagent.\cite{28}

The composition of major phenolic substances was determined by HPLC - PDA analysis HPLC system. HPLC Column: LC-C18 reversed-phase column (25 cm x 4.6 mm, 5 mm; Sigma), injection volume: 50 µl using auto sampler, Detector: PDA detector (model 1260 infinity) set at 280 and 340 nm .Pump condition: gradients condition were formed by the quaternary pumping system by varying the proportion of flow rate and solvent conditions from solvent A: water–acetic acid (97:3, (v/v)) to solvent B (methanol). The typical chromatogram recorded at 280 and 340 nm. phenolic acids and flavonoids were tentatively identified and quantified in the investigated fruits and seeds by comparison with the retention times and UV spectra of standards that were analyzed under identical conditions.

Ultra-Violet Spectrophotometric analysis

Chromatographically, pure materials dissolved in analytically pure methanol were subjected to UV Spectrophotometric investigation in 4 ml capacity quartz cells Zeiss spectrometer PMQ-II. In case of flavonoids, AlCl3, AlCl3 /HCl, fused NaOAc /H3Bo3 and NaOMe reagents were separately added to methanolic solution of the investigated material and UV measurements were then carried out.\cite{29}

Identification of Hydrocarbon and fatty acids methyl esters in seed extract determined by gas chromatography (GC)-Mass system

The analytical GC-MS analyses were performed in two different equipments: (a) Hewlett Packard 5973–6890 system, operating on EI mode and equipped with a HP 5MS 30 m x 0.25 mm×0.25 µm film thickness capillary column. The carrier gas was Helium (flow rate=1 mL/min). Temperature program: initial column temperature 60 °C (for 5 min), raised to 280 °C within 3 °C/min, and held there for 15 min. The injector and detector temperatures were 220 and 280 °C, respectively, (b) Finnigan trace GC ultra system operating on EI mode and equipped with
**AT™ Aquawax 30 m×0.32 mm×0.25 μm film thickness capillary column. The carrier gas was Helium (flow rate=1.5 mL/min, constant flow) and Split ratio, 1:10.**

**Experimental animals and design**

Forty two male Sprague Dawely rats weighing 180-200 g (aged 3-4 months) were enrolled in this study. Rats were bred and housed in the animal house of the Research Center, Faculty of Science Zagazig University, Egypt. Rats were housed in glass cages and kept under environmentally controlled conditions with a 12 hour light/dark cycle and have free access to the tape water. All experimental procedures and techniques were performed according to the international guidelines for animal ethics and care and approved by local ethical committee of Zagazig Faculty of Science, Egypt.

Rats were randomly divided into equal six groups (7 rats each); a) normal control (NC) group; normal rats, b) Diabetic control (DC) group; rats feed on high fat diet for 2 weeks, then received STZ (35 mg/kg) and received saline for 8 weeks c) DM + AFE group: as control group with aqueous Fruit extract (4 ml /kg BW) for 8 weeks, d) DM + MFE group: as control group with methanolic fruit extract (1mg/Kg BW) for 8 weeks, e) DM + AFE group: as control group with aqueous seed extract (10 ml /kg BW) for 8 weeks and f) DM + AFE group: as methanolic seed extract (2 mg/kg BW) aqueous seed extract.

**Induction of type 2 DM rat model**

Type 2 DM was induced according the technique done by Hussein et al.,\[30]\ Briefly, rats feed on a high-fat diet consisting of 22.5% commercially available hydrogenated vegetable oil, 22.5 % milk powder, 51.5 % soybean ground, 2 % corn starch, 1 % sucrose and 0.5 % vitamins and minerals for two weeks, then rats were injected with 35 mg/kg STZ (Sigma chemical Company, Saint Louis, MO, USA) freshly prepared in cold 0.1 mol citrate buffer (pH 4.5) in tail vein after overnight fasting. Hyperglycemia was confirmed two days after STZ injection by detection of glucose in urine by glucose strips, then blood glucose was measured and rats with blood glucose levels more than 250 mg/dl (in two successive samples) were used in this study.

**Collection of blood samples and liver specimens**

At the end of experiment (8 weeks of treatment), rats were weighed and blood samples were obtained from the ophthalmic venous plexus using fine–walled Pasteur pipette under halothane anaesthesia, then animals were sacrificed by an overdose of Na⁺-thiopental (75
mg/kg b.w., i.p.), then the abdomen was opened liver was harvested and washed with phosphate-buffered saline (PBS) to rinse out the blood. Liver was rapidly placed in a container containing 10% neutral buffered formalin for histopathological examination. Blood samples were centrifuged at 1000 rpm and serum stored at -20°C till the time of biochemical analysis.

**Measurement of serum glucose, albumin, AST, ALT and bilirubin**

These markers were measured by specific kits according to manufacturer instructions. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, bilirubin and glucose were measured and analyzed using Human (Germany) and Randox (United Kingdom) Diagnostic kits /RoboniK (India) instrument.

**Histopathological examination**

The liver specimen was embedded in paraffin, sliced into 5 μm sections, and stained with hematoxylin-eosin for blinded histological examination. Liver sections were examined for inflammatory cells, hepatocytes necrosis and macrovesicular fatty changes in centrilobular zone.

**Statistical analysis**

All data were expressed as mean ± standard deviation (SD). ANOVA with Tukey’s post-hoc test was used for repeated measures, comparison. P value ≤ 0.05 was considered as statistically significant. All analyses were carried out using the SPSS computer program version 14.0 for windows.

**RESULTS AND DISCUSSION**

**Saturated and unsaturated long chain hydrocarbons in n-hexan extracts identified by GC-MS**

These compound obtained from seed extracts by GC-mass analysis had been showed in fig (1) table (1). They were Benzene,1,2dimethyl,Benzene, ethyl, p-Xylene, 1,3,5-Cycloheptatriene,7-ethyl,Butylated hydroxytoluene, Octadecane, 6-methyl, Octadecane, 1chloro,9-Octadecenoic acid ,Diisooctyl phthalate, 3a-Cholest-5-en-3 ol, hexadecadienoicacid, methylester, ethyl isoalchalolate and Quercetin7,3’,4’trimethoxy. These findings were in agreement with Al-Shahib and Marshall[31] who found that the oleic acid (9-Octadecenoic acid) content of 24 cultivars of the date seed oil ranges from 41 to 59%, which could be a good source of C18:1 fatty acid. Also, these findings are in agreement with Besbes et al.,[32] Besbes et al.,[33] Nehdi et al.,[34] Rahman et al.,[35] Al-Shahib and Marshall.[36]
who detected that date seed oil is mainly composed of the four fatty acid namely oleic, linoleic, lauric and palmitic acid.

**Isolation and identification of phenolic compounds in aqueous and methanolic fruit extracts**

The HPLC chromatograms for methanolic and aqueous extract of palm date frits extract. figure(2,3) was identified eight phenolic and flavonoids glycosides by analyses.(Caffeic acid, p-Coumaric acid, Ferulic acid, Chlorogenic acid, Sinapic acid, Luteolin- 7- O- rutinoside, Apiginin-c-glycoside and Quercetin-3-O-rutinoside). This finding is in agreement with Mansouri et al.\[37\] who analyzed phenolic profile of seven Algerian varieties of date and observed that they contain p-coumaric, ferulic and sinapic acids, some cinnamic acid derivatives and three different isomers of 5-o-caffeoyl shikimic acid. Also, these findings are in line with those reported by Hong et al.,\[38\] who indicated that quercetin and luteolin form primarily O-glycosidic linkages, whereas apigenin is present as the C-glycoside in Mature (khalal stage) of Deglet Noor dates.

**Isolation and identification of phenolic compounds in aqueous and methanolic seed extracts**

HPLC chromatograms for methanolic and aqueous extracts of palm date seed produced five phenolic compounds (vanillic acid, gallic acid, caffeic acid, p-coumaric acid and quercitin), while fruits extracts produced 8 compounds (caffeic acid, p-coumaric acid, ferulic acid, chlorogenic acid, sinapic acid, luteolin- 7-O-rutinoside, apiginin-c-glycoside and quercetin-3-O-rutinoside (fig.2 and 3). This is in agreement with Al-Farsi and Lee,\[12\] who detect phenolic acids in date seed such as gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, m-coumaric acid and o-coumaric acid. Also, Ammar et al.\[39\] reported similar results for phytochemicals on seed of the plant. Moreover, Mansouri et al.,\[37\] analyzed phenolic profile of seven Algerian varieties of date and observed that they contain p-coumaric, ferulic and sinapic acids, some cinnamic acid derivatives and three different isomers of 5-o-caffeoyl shikimic acid.

**Biological effects of methanolic and aqueous extracts of fruits and seeds in DM**

Fasting blood glucose showed significant increase in DC group compared to NC group (p< 0.001) and this increment in fasting blood glucose was significantly attenuated in other studied (DM+AFE, D.M+MFE, D.M+ASE and D.M+MSE) groups. Treatment with both types of fruit extracts (AFE, MFE) of fruits showed significant decrease in fasting blood
glucose compared to seed extracts (ASE, MSE) especially methanolic extracts (p< 0.05) (table 1). This result is in agreement with Zangiabadi et al.[40] who demonstrated that treatment with date fruit aqueous extract improved fasting blood sugar and prevent diabetes hazards and cause improvement in diabetic neuropathy in STZ-induced diabetic rats. Also, Mokhtari et al.[41] showed that alcoholic extract of seeds of dates decreased the blood glucose in male diabetic rats.

Also, the hepatoprotective effects of palm dates demonstrated in the present study were shown in table 2 and fig.4. Liver enzymes (ALT&AST) were significantly high in diabetic group compared to normal group (p < 0.001). Treatment with either seed or fruit extracts significantly attenuated liver enzymes compared to DC group (p < 0.05). On the other hand, serum albumin was significantly low in DC group compared to normal group (p < 0.001) and treatment with all types of extracts caused significant improvement in serum albumin (p < 0.05) and the improvement was significantly high in AFE group. Lastly, serum bilirubin showed no significant change among all groups. These findings suggest hepatic injury in type 2 DM and the hepatoprotective effect for palm date extracts.

Al- Qarawi et al.[42] evaluated ameliorative activity of aqueous extracts of flesh and pits of dates in CCl4 induced hepatotoxicity rat model. They demonstrated similar hepatoprotective effects for the palm date extracts on CCL4 –induced hepatotoxicity (p<0.001). Also, Chukwugozie et al.,[43] showed significant rise in the level of biochemical makers of liver damage like ALT, AST, ALP and total bilirubin and a fall in albumin in thioacetamide -treated groups when compared with the respective values for normal rats. Moreover, palm date extracts showed significant reduction in liver enzymes and elevation in serum albumin (p<0.05). This hepatoprotective effect for palm date extracts could be attributed to its contents such as quercetin which has a strong antioxidant effects.

Fig 5 represents liver samples obtained from different groups. At the level of liver morphology, liver sections obtained from DM group showed fatty changes in centrilobular portions of the livers. These results are in line with the findings reported by Ramesh et al.,[44] and Abolfathi et al.,[45] who observed considerable fatty change in liver of STZ-induced diabetic rats. Also, the present study showed significant improvement in liver morphology by both aqueous and methanolic fruit and seed extracts. Rats treated with aqueous fruit extracts showed nearly normal liver architecture.
In conclusion, this is the first study to demonstrate the hepatoprotective action of aqueous and methanolic extracts of palm date fruits and seeds against hepatic dysfunction in type 2 DM. In this work, we identify the different phytochemicals present in the palm date fruits and seeds by HPLC analysis and GC-MS. This study shows the richness of aqueous and methanolic palm dates extracts in various chemical groups having an antioxidant capacity which offers beneficial purposes such as flavonoids glycosides, phenolic acids were determined HPLC method. This hepatoprotective action according to improvement of glucose level, also investigated the possible protective effect for antioxidant of aqueous and methanolic extracts of palm date fruit and seed such as quercetin, p-coumaric acid, ferulic acid, caffeic, and chlorogenic acid. The results presented her suggest that date palm fruit serves as a good source of active components characterized by beneficial effect on a large number of diseases.

Table (1): Compounds identified by GC/Ms of crude extract of n-hexan (25ºc) extract of the date palm seed (Phoenix dactylifera)

<table>
<thead>
<tr>
<th>Chemical Composition</th>
<th>Compound Structure</th>
<th>Hit Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene, 1,2dimethyl</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>Benzene, ethyl</td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>p-Xylene</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>1,3,5-Cycloheptatriene, 7 ethyl</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
<tr>
<td>Compound</td>
<td>Structures</td>
<td>Spectra</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td>Butylated Hydroxytoluene</td>
<td><img src="structure1.png" alt="Butylated Hydroxytoluene Structure" /></td>
<td><img src="spectrum1.png" alt="Butylated Hydroxytoluene Spectrum" /></td>
</tr>
<tr>
<td>Octadecane, 6-methyl</td>
<td><img src="structure2.png" alt="Octadecane, 6-methyl Structure" /></td>
<td><img src="spectrum2.png" alt="Octadecane, 6-methyl Spectrum" /></td>
</tr>
<tr>
<td>Octadecane, 1-chloro</td>
<td><img src="structure3.png" alt="Octadecane, 1-chloro Structure" /></td>
<td><img src="spectrum3.png" alt="Octadecane, 1-chloro Spectrum" /></td>
</tr>
<tr>
<td>9-Octadecenoic acid</td>
<td><img src="structure4.png" alt="9-Octadecenoic acid Structure" /></td>
<td><img src="spectrum4.png" alt="9-Octadecenoic acid Spectrum" /></td>
</tr>
<tr>
<td>Diisooctyl phthalate</td>
<td><img src="structure5.png" alt="Diisooctyl phthalate Structure" /></td>
<td><img src="spectrum5.png" alt="Diisooctyl phthalate Spectrum" /></td>
</tr>
<tr>
<td>(3a)-Cholest-5-en-3-ol</td>
<td><img src="structure6.png" alt="Cholest-5-en-3-ol Structure" /></td>
<td><img src="spectrum6.png" alt="Cholest-5-en-3-ol Spectrum" /></td>
</tr>
<tr>
<td>Hexadecadienoic acid, methyl ester</td>
<td><img src="structure7.png" alt="Hexadecadienoic acid, methyl ester Structure" /></td>
<td><img src="spectrum7.png" alt="Hexadecadienoic acid, methyl ester Spectrum" /></td>
</tr>
</tbody>
</table>
Table (2): Effects of different extracts on liver function parameters (serum bilirubin, albumin, AST and ALT)

<table>
<thead>
<tr>
<th>group</th>
<th>Serum bilirubin (mg/dl)</th>
<th>Serum albumin (gm/dl)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC (n=7)</td>
<td>0.5 ± 0.11</td>
<td>4.51 ± 0.36</td>
<td>28.7 ± 3.03</td>
<td>26.28 ± 2.13</td>
</tr>
<tr>
<td>D.M(n=7)</td>
<td>0.59 ± 0.1</td>
<td>1.32 ± 0.17*</td>
<td>55.85 ± 3.53*</td>
<td>47.71 ± 3.09*</td>
</tr>
<tr>
<td>DM+AFE(n=7)</td>
<td>0.62 ± 0.16</td>
<td>3.75 ± 0.13*</td>
<td>27.1 ± 3.13*</td>
<td>27.42 ± 3.50*</td>
</tr>
<tr>
<td>D.M+MFE(n=7)</td>
<td>0.62 ± 0.14</td>
<td>3.14 ± 0.15*</td>
<td>26.14 ± 4.48*</td>
<td>27.0 ± 2.3*</td>
</tr>
<tr>
<td>D.M+ASE(n=7)</td>
<td>0.68 ± 0.1</td>
<td>2.52 ± 0.11*</td>
<td>24.28 ± 3.49*</td>
<td>25.85 ± 3.97*</td>
</tr>
<tr>
<td>DM+MSE(n=7)</td>
<td>0.64 ± 0.29</td>
<td>1.94 ± 0.17*</td>
<td>25.57 ± 3.64*</td>
<td>25.14 ± 3.62*</td>
</tr>
</tbody>
</table>

All data were expressed in mean ± SD. * significant vs. NC group, # significant vs. DM group, $ significant vs. DM+ AFE group. NC: Normal control, DC: diabetic control, DM+AFE: diabetic rats treated with aqueous Fruit extract (4ml/kg BW), D.M+MFE: diabetic rats treated with methanolic fruit extract (1 mg/kg BW), D.M+ASE: diabetic rats treated with aqueous seed extract (10ml/kg BW), D.M+MSE: diabetic rats treated with methanolic seed extract (2 mg/kg BW).

Fig. (1): Typical GC- mass chromatogram of separated hydrocarbon
Fig (2): HPLC chromatography of methanolic and aqueous extracts of the date palm fruits (Phoenix dactylifera) at 280 nm (a) – 340 nm (b).

Fig (3): HPLC chromatography of methanolic and aqueous extracts of the date palm seeds (Phoenix dactylifera) at 280 nm (a) – 340 nm (b).
Fig. (4). The effect of different palm date fruits and seed extracts on blood glucose.

*significant vs. NC group, #significant vs. DM group, §significant vs. DM+ AFE group,

§significant vs. DM+MFE and †significant vs group treated with ASE. NC: Normal control, DC: diabetic control, DM+AFE: diabetic rats treated with aqueous Fruit extract (4ml/kg BW), D.M+MFE: diabetic rats treated with methanolic fruit extract (1 mg/kg BW), D.M+ASE: diabetic rats treated with aqueous seed extract (10ml/kg BW), D.M+MSE: diabetic rats treated with methanolic seed extract (2 mg/kg BW).
Fig. (5): Liver specimens showing a) normal liver with the characteristic pattern of the hepatocytes trabeculae between central veins and portal areas (white arrows) (NC group, H&E X200), b) injured liver with hepatocytes necrosis and macrovesicular fatty changes around CV with areas of haemorrhage and inflammatory cells in portal tract (black arrows) (DM group, H&E X200), c) nearly normal liver with normal architecture of hepatocytes plates and portal tract (DM+ AFE group, X200), d) liver with focal degenerative changes in the form of hyperchromatic large nuclei and frequent mitosis (white arrows) (DM+ MSE group, H&E X400).

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REFERENCES


