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Antidiabetic, Antihyperlipidemic and Antioxidant Activities of *Acacia albida* in Streptozotocin-induced Diabetes in Rats and its Metabolites

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THE particular purpose of this research was to assess the antidiabetic, antihyperlipidemic and antioxidant activities of *Acacia albdia* methanol extract. In addition, a phytochemical study for *A. albdia* has been performed. Isolation and identification of pure compounds were carried out by different chromatographic and spectroscopic techniques. Quercetin, kaempferol and their glycosides have been isolated from *A. albida* leaves. Structures of the isolated compounds have been estimated by 1D/2D 1H/13C-NMR spectroscopy. Methanolic extract of the leaves of *A. albida* improved the diabetes status, and it showed antidiabetic, antihyperlipidimic and antioxidant effects. These findings suggest that *A. albida* leaves may be used as hypolipidemic and normo-glycemic agents, additionally; *A. albida* is a rich source of phenolic compounds.

Keywords: Acacia albida, Antidiabetic, Antihyperlipidimic, Antioxidant, Triglycerides.

Introduction

Diabetes mellitus is an epidemic chronic disease affecting nearly about 5% of the world's population and is triggered by hereditary or acquired by deficiency in insulin secretion and reduced organ response to insulin secretion[1-3]. Diabetes is a chronic form that occurs when the body cannot create enough insulin or utilize it appropriately. In this condition, the organism can produce insulin but becomes resistant to it, causing the insulin to be ineffective. High blood glucose levels are a result of insulin resistance and insulin deficiency. Therefore, elevations of blood glucose levels are a result of insulin resistance and insulin shortage. Type 2 diabetics suffer from insulin resistance and generally relative rather than absolute insulin deficiency[4, 5].

The treatment of diabetes without any side impacts remains a challenge in the therapeutic field as such renewed attention has been paid for alternative remedies and natural therapies[6,7]. Natural products have become a destination to find new remedies and biological active "lead" compounds[8]. Ethnobotanical data showed that more than 800 plants are used as a therapy of diabetes as traditional remedies[9,10], but only a few of them have been scrutinized scientifically. People around the world relied on herbs to treat various diseases before the appearance of modern medicine. Today many plants are used in Egypt's folk medicine and are sold at herbal shops [11,12]. The ancient Egyptians knew about various medicinal herbs and their use to treat different diseases, as they used the plant organs;

roots, flowers, rhizomes, fruits, seeds, leaves, and oils. They used their medicaments in the form of pills, powders, creams suppositories, ointments and pastes [13].

The genus *Acacia* includes approximately 1350 species [14], distributed in tropics and to some extent in the temperature areas. It is widely distributed throughout the dry zones of tropical Africa. The main area of natural distribution of *Acacia albida* is Africa, and ranges from Senegal and Gambia to the Red Sea (Egypt, Sudan, Ethiopia, Somalia, and Kenya), across the African continent [15]. *A. albida* is distinctive of shedding its leaves at the beginning of the rainy season, and of turning into foliage in the dry season. *A. albida* possess antimicrobial, and antiviral effects[16-17]. It is also used in the treatment of diarrhea, stomachache, haemorrhage cough, pneumonia postpartum complications, and renal diseases[18]. The current study was undertaken to determine whether the pancreas is exposed to oxidative damage during diabetes, and examine the associated modifications in the status of antioxidant to clarify its role in the pathogenesis of the disease. In addition, we investigated whether the treatment with *A. albida* protects against damage of pancreatic-cell in STZ-induced diabetic rats.

Materials and Methods

General procedures

NMR experiments were performed on a Bruker AMX 400 and 500 instruments with standard pulse sequences operating at 400, 500 MHz in ^{1}H NMR and 100, 125 MHz in ^{13}C NMR. Chemical shifts were given in δ values (ppm) using TMS as the internal standard and DMSO-d_6 as solvent at room temperature. HRESI-MS was taken on a Micromass Autospec (70 eV) spectrometer. UV spectral data was measured on a Shimadzu 240 spectrometer in MeOH. Paper chromatography was run on Whatman sheets No. 1, using A: 15% AcOH and B: n-BuOH: AcOH: H_2O (4:1:5, upper layer) as the solvent systems. Compounds were visualized by exposure to UV light (365 nm), before and after spraying with AlCl$_3$ and Naturstoff polyethylene glycol reagents.

Plant materials

Leaves of *A. Albida* were collected in April 2015 from the Upper Egypt. Identification of the plants was confirmed by Prof. Dr. Ibrahium El-Garf, Department of Botany, Faculty of Science, Cairo, University and comparison with herbarium

specimens. Voucher Specimens were kept in herbarium, Department of Botany, Faculty of Science, Cairo University[19].

Extraction and isolation

Air dried ground leaves of *A. Albida* (1.5kg) were defatted with petroleum ether (40-60°C, 3 x 2.5L), and extracted three times at room temperature with C_2H_5OH-H_2O (7:3). The combined extracts were filtered, then evaporated under reduced pressure and lyophilized (180 g). Twenty grams of the dry residue were used for Pharmacological studies. Weighed samples of the extract were used to prepare the solutions by dilution with distilled H_2O to the appropriate concentration for the experiment. The rest of the dry extract was re-dissolved in 2 L H_2O and extracted with n-butanol (3 x 2 L). After evaporation of solvents, the n-butanol extract and the remaining H_2O phase gave dark brown solids 50 and 70 g, respectively. The n-butanol extract was loaded on a polyamide 6S column chromatography (80x3 cm). The column was eluted with H_2O, and then H_2O-CH_3OH mixtures of decreasing polarity and 10 fractions (1L, each) were collected. The major flavonoids fractions were combined into five fractions after chromatographic analysis. Fraction 1 (2.5 g) was fractionated by column chromatography on Sephadex LH-20 with aqueous EtOH (0- 70%) for elution to give compounds **1** (17 mg) and **3** (25 mg). Fraction 2 (1.6 g) was subjected to column chromatography on cellulose and n-BuOH saturated with H_2O as an eluent to give two major subfractions, then one of them was separately fractionated on a Sephadex LH-20 to yield pure sample of **2** (22 mg). Using the same procedure fraction 3 (1.8 g) and fraction 4 (1.4 g) gave chromatographically pure samples of **4** (15 mg) and **5** (15 mg). Fraction 5 (1.5 g) was chromatographed on Sephadex LH-20 using aqueous acetone (0-25%) for elution to give pure samples of **6** (20 mg) and **7** (25 mg).

Spectroscopic data

Quercetin-3-*O*-β-glucopyranoside (1): Yellow amorphous powder; UV λmax nm: (MeOH) 253, 263sh, 294 sh, 352; (MeOH+ NaOMe) 271, 328sh, 410; (MeOH + NaOAc) 273, 321, 375; (MeOH+NaOA+H_3BO_3) 262, 300sh, 377; (MeOH+AlCl$_3$) 275, 305sh, 332sh, 435; (MeOH+AlCl$_3$+HCl) 275, 305sh, 361sh, 403. ^{1}H NMR (DMSO-d_6): δ 7.67 (1H, dd, J = 2.1 and 8.6 Hz, H-6′),7.53 (1H, d, J = 2.1 Hz, H-2′), 6.82 (1H, d, J = 8.6 Hz, H-5′), 6.40 (1H, d, J = 1.8 Hz, H-8), 6.20 (1H, d, J = 1.8 Hz, H-6), 5.37 (1H, d, J = 7.6

Hz, H-1″), 3.28-3.65 (m, rest of glucose protons). ^{13}C NMR: δ 156.80 (C-2), 133.60 (C-3), 177.50 (C-4), 161.60 (C-5), 98.90 (C-6), 164.60 (C-7), 93.80 (C-8), 156.60 (C-9), 104.00 (C-10), 121.60 (C-1′), 115.80 (C-2′), 145.80(C-3′), 148.80 (C-4′), 116.20 (C-5′), 122.00 (C-6′), 101.20 (C-1″), 71.60 (C-2″), 74.40 (C-3″), 70.02 (C-4″), 77.70 (C-5″), 61.50 (C-6″).

Kaempferol-3-O-β-glucopyranoside (2): Greenish yellow amorphous powder, UV λmax nm: (MeOH) 256, 292 sh, 357; (MeOH+ NaOMe)272, 325sh, 415; (MeOH + NaOAc) 273, 315, 390; (MeOH+NaOA+H$_3$BO$_3$) 261, 320sh,377; (MeOH+AlCl$_3$) 274, 340sh, 430; (MeOH+AlCl$_3$+HCl) 269, 340sh, 400.^{1}H NMR (DMSO-d_6): δ 7.92 (2H, d, J = 8.1 Hz, H-2′,6′), 6.88 (2H, d, J = 8.1 Hz, H-3′,5′), 6.36 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8 Hz, H-6), 5.33 (1H, d, J = 7.6 Hz, H-1″), 3.28-3.65 (m, rest of glucose protons). ^{13}C NMR: δ 156.76 (C-2), 133.57 (C-3), 177.82 (C-4), 161.59 (C-5), 99.10 (C-6), 164.63 (C-7), 94.04 (C-8), 156.62 (C-9), 104.34 (C-10), 121.28 (C-1′), 131.24 (C-2′,6′), 115.47 (C-3′,5′), 160.31 (C-4′), 101.29 (C-1″), 74.59 (C-2″), 76.80 (C-3″), 70.28 (C-4″), 77.83 (C-5″), 61.28 (C-6″).

Quercetin-3-O-α-L-rhamnopyranoside (3): Yellow powder; UV λmax nm: (MeOH) 253, 263sh, 344; (MeOH+ NaOMe) 272, 322sh, 372; (MeOH+ NaOAc) 260, 300sh, 367; (MeOH+ NaOAc + H$_3$BO$_3$) 272, 382; (MeOH+AlCl$_3$) 272, 304sh, 333sh, 430; (MeOH+AlCl$_3$ +HCl) 272, 303sh, 353, 401. ^{1}H NMR (DMSO-d_6): 7.33 (1H, d, J = 2 Hz, H-2′), 7.28 (1H, dd, J = 2 Hz, and J = 8.5 Hz, H-6′), 6.90 (1H, d, J =8.5 Hz, H-5′), 6.42 (1H, d, J = 2 Hz, H-8), 6.23 (1H, d, J = 2 Hz, H-6), 5.29 (1H, d, J = 1.4 Hz, H-1″), 3.16-3.56 (m, rest of rhamnose protons), 0.85 (d, J = 6.07 Hz, CH$_3$ of rhamnose). ^{13}C NMR: δ 157.34 (C-2), 134.27 (C-3), 177.79 (C-4), 161.35 (C-5), 98.78 (C-6), 164.34 (C-7), 93.70 (C-8), 156.86 (C-9), 104.16 (C-10), 121.17 (C-1′), 115.52 (C-2′), 145.25 (C-3′), 148.50 (C-4′), 115.72 (C-5′), 120.80 (C-6′), 101.87 (C-1″), 70.43 (C-2″), 70.63 (C-3″), 71.25 (C-4″), 70.11 (C-5″), 17.54 (C-6″).

Kaempferol-3-O-α-L-rhamnopyranoside (4): Yellow amorphous powder. UV λmax nm: (MeOH) 256, 292 sh, 357; (MeOH+ NaOMe) 272, 325sh, 415; (MeOH + NaOAc) 273, 315, 390; (MeOH+NaOA+ H$_3$BO$_3$) 261, 320sh, 377; (MeOH+AlCl$_3$) 274, 340sh, 430; (MeOH+AlCl$_3$+HCl) 269, 340sh, 400: ^{1}H NMR (DMSO-d_6): δ 7.92 (2H, d, J = 8.1 Hz, H-2′,6′),

6.88 (2H, d, J = 8. 1 Hz, H-3′,5′), 6.36 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8 Hz, H-6), 5.33 (1H, d, J = 7.6 Hz, H-1″ of glucose), 3.28-3.65 (m, rest of glucose protons). ^{13}C NMR: δ 156.76 (C-2), 133.57 (C-3), 177.82 (C-4), 161.59 (C-5), 99.10 (C-6), 164.63 (C-7), 94.04 (C-8), 156.62 (C-9), 104.34 (C-10), 121.28 (C-1′), 131.24 (C-2′,6′), 115.47 (C-3′,5′), 160.31 (C-4′), 101.87 (C-1″), 70.43 (C-2″), 70.63 (C-3″), 71.25 (C-4″), 70.11 (C-5″), 17.54 (C-6″).

Quercetin-3-O-α-L-arabinoside (5): Yellow amorphous powder. UV λmax (nm) (MeOH): 260, 300sh, 362;(MeOH + NaOMe) 277, 333, 412; + NaOAc: 276, 325, 388;(MeOH + NaOAc /H$_3$BO$_3$) 262, 300sh, 382; (MeOH +AlCl$_3$) 276, 303, 338sh, 438;(MeOH + AlCl$_3$/HCl) 276, 303sh, 367, 405; Negative FAB-Mass: [M-H]= m/z 433; ^{1}H-NMR: δ (ppm) 7.64 (1H, d, J = 2.1 Hz, H-2′), 7.49 (1H,dd, J = 2.1 Hz and J = 8.4 Hz, H-6′), 6.82 (1H, d, J = 8.4 Hz, H-5′), 6.39 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8 Hz, H-6); 5.26 (1H, d, J = 5.13 Hz, H-1″), 3.12-3.63 (m, rest of arabinose protons); ^{13}C-NMR: δ (ppm) 156.63 (C-2), 134.05 (C-3), 177.84 (C-4), 161.53 (C-5), 99.05 (C-6), 164.59 (C-7), 93.90 (C-8), 156.63 (C-9), 104.22 (C-10), 121.22 (C-1′), 115.71 (C-2′), 145.31 (C-3′), 148.94 (C-4′), 116.10 (C-5′), 122.36 (C-6′); 101.76 (C-1″), 71.07 (C-2″), 72.00 (C-3″), 66.46 (C-4″), 64.70 (C-5″).

Quercetin (6): Yellow amorphous powder, UV λmax nm: (MeOH) 253, 268sh, 297sh, 368; (MeOH$^+$NaOMe) 247sh, 321. (dec.); (MeOH + NaOAc) 257sh, 274, 329, 390. (dec.); (MeOH+NaOA+H$_3$BO$_3$) 261, 303sh, 388; (MeOH+AlCl$_3$) 272, 304sh, 333, 458; (MeOH+AlCl3+HCl) 265, 301sh, 359, 428.: ^{1}H NMR (DMSO-$d6$): δ 7.69 (1H, d, J = 2.1, H-2′), 7.55 (1H, dd, J = 2.1 Hz, and 8.4 Hz, H-6′), 6.90 (1H, d, J = 8.4 Hz, H-5′), 6.42 (1H, d, J = 1.8 Hz, H-8), 6.20 (1H, d, J = 1.8 Hz, H-6).^{13}C NMR: δ 147.50 (C-2), 136.44 (C-3), 176.55 (C-4), 161.43 (C-5), 98.88 (C-6), 164.59 (C-7), 94.05 (C-8), 156.83 (C-9), 103.71, (C-10), 122.66 (C-1'), 116.31 (C-2′), 145.76 (C-3′), 148.40 (C-4′),115.76 (C-5′), 120.68 (C-6′).

Kaempferol (7): Greenish yellow powder, UV λmax nm: (MeOH) 254 sh, 268, 322sh, 365; (MeOH+ NaOMe), 275, 320, 412 (dec.); (MeOH + NaOAc), 275, 300, 385; (MeOH+NaOA+ H$_3$BO$_3$) 269, 295sh, 370; (MeOH+AlCl3) 262 sh, 270, 353, 426; (MeOH+AlCl$_3$+HCl) 260, 271, 350, 428. ^{1}H NMR (DMSO-d_6): δ 7.9 (2H, d, J = 8.1, H-2′, 6′); 6.90 (2H, d, J = 8.2 Hz, H-3′, 5′),

6.42 (d, J = 2.4 Hz, H-8), 6.20 (1H, d, J = 2.4 Hz, H-6). ^{13}C NMR: δ 146.80 (C-2), 135.84 (C-3), 176.20 (C-4), 161.60, (C- 5), 98.60 (C-6), 164.59 (C-7), 93.85 (C-8), 156.40 (C-9), 103.70, (C-10), 121.90 (C-1'), 129.90 (C-2', C-6'), 115.80 (C-3', C-5'), 159.5 (C-4').

Experimental animals

Thirty-six female albino rats (110-130 g) were obtained from the Lab Animals House, National Research Centre, Cairo, Egypt. All the animals were maintained under laboratory conditions of temperature (22±2°C), humidity (45±5%) and 12 h day: 12 h night cycle and were allowed free access to food (standard pellet diet) and water *ad libitum*.

Induction of diabetes

Diabetes was induced in 24 fasted rats by intraperitoneal (*i.p.*) injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in 0.1 M cold citrate buffer (pH = 4.5) [18,19]. Diabetes was stabilized in these STZ treated rats over a period of 72 hours. After this time, the blood was collected by sinocular puncture and the plasma glucose level of each rat was determined. Rats with a fasting plasma glucose range of ≥ 250 mg/dl were considered diabetic and included in the study[20].

Experimental design and treatment schedule

Rats were randomly divided into six groups; six rats each as follow :

Group 1: Non-diabetic control rats treated with distilled water.

Group 2: Diabetic control rats treated with distilled water.

Group 3: non-diabetic rats treated with 100 mg/kg of *A. albida*.

Group 4: non-diabetic rats treated with 200 mg/kg of *A. albida*.

Group 5: diabetic rats treated with 100 mg/kg of *A. albida*.

Group 6: diabetic rats treated with 200 mg/kg of *A. albida*.

Treatment with *A. albida* was administered orally using an intra-gastric tube once daily for 21days.

Biochemical assays

At the end of the experiment, rats were fasted overnight and blood samples were withdrawn through the retro-orbital plexus under light ether anesthesia using a heparinized glass capillary and collected in tubes. Blood was allowed to clot and serum separated by centrifugation at 4000 rpm

for 10 min. The livers were removed and divided into two parts, first part washed with ice-cold saline solution (0.9% NaCl), weighed and stored at -80ºC for the biochemical analyses. Tissues were homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10% w/v for the biochemical assays. The second part of liver and pancreas were fixed in formalin saline mixture for pathological study. Serum glucose, and serum insulin (using Ins-Elisa kit, Biosoure Brussels Belgium) were estimated by the methods of Trinder and Yalow & Bauman [21,22], respectively, and Insulin resistance was calculated according to the method described by Matthews[23], as follows:

$$\text{Insulin resistance} = X \frac{\text{Fasting blood glucose (mg/dl)} \times \text{fasting insulin (}\mu\text{IU/ml)}}{405}$$

Total cholesterol, triglycerides, and HDL were estimated according to the methods of Flegg[24], Fredrickson [25], and Finley [26], respectively. LDL was calculated as follows :

LDL= total cholesterol - HDL - TG/5[27].

Coenzyme Q10 measurement was performed using HPLC assay as described by Mosca[28]. Malondialdehyde was determined in liver homogenate calorimetrically[29]. Q10 levels in tissues were measured according the methods of Tang, et al., [30].

Histopathological studies

Liver and pancreas samples were excised, washed with normal saline and processed separately for histopathological observation. In the beginning, the materials were fixed in 10% buffered neutral formalin for 24h, and dehydrated in ascending series of ethanol, After that, embedded in paraffin, sectioned at 5µm thickness, and stained with alum hematoxylin and eosin The sections were examined microscopically for histopathology changes.

Statistical analysis

All values obtained were evaluated as mean ± standard error and the statistical significance of differences between mean results was determined using the SPSS for Windows version 11.0 statistical program (Chicago, IL, USA). The parametric continuous variables were evaluated with the one-way ANOVA test. Study groups were compared with the control group by applying the test. For all statistical evaluations, *P* values less than 0.05 were recognized as statistically significant.

Results and Discussion

Phenolic compounds of Acacia albida

Extract fractionation led to isolate and identify seven flavonoids **(1-7)** (Fig. 1). Structures of these compounds were confirmed with chemical and spectroscopic analysis (UV, 1/2D NMR)[31-33].

Biochemical results

The serum glucose and insulin levels of normal and experimental animals were shown in Table 1. Serum glucose levels and insulin resistance were elevated significantly ($P<0.05$), while there was a significant decrease in insulin level ($P<0.05$) in the diabetic control animals relative to non-diabetic control group. During the experiment, administration of *A. albida* at 100 and 200 mg/kg body weight tended to attain serum glucose, insulin resistance and insulin to normal values, while normal rats showed no important changes in insulin resistance, serum glucose levels and insulin concentration. From Table 2, there was a substantial decrease in serum HDL-cholesterol

level and a substantial elevation in total cholesterol, LDL-cholesterol and triglycerides levels in diabetic groups relative to normal ones ($P<0.05$). Administration of *A. albida* at the doses of 100 and 200 mg/kg body weight for 21 days attained back the serum lipids nearly to normal. The administration of *A. albida* at the doses of 100 and 200 mg/kg body weight showed nonsignificant change serum levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in normal rats. Table 3 presented the effect of the administration of *A. albida* on liver malondialdehyde and serum Q10 in normal and diabetic rats. In diabetic rats, Q10 decreased significantly ($P<0.05$) and liver malondialdehyde increased significantly ($P<0.05$) compared to the normal rats. Oral administration of *A. albida* in both doses for 21 days returned the levels of Q10 and malondialdehyde to the normal levels. While normal rats showed no significant alternations in serum Q10 and liver malondialdehyed rates during the experiment.
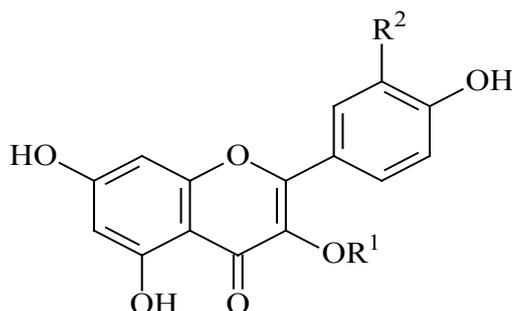
R$_2$	R$_1$	No.	R$_2$	R$_1$	No.
OH	arabinoside	**5**	OH	glucoside	**1**
OH	H	**6**	H	glucoside	**2**
H	H	**7**	OH	rhamnoside	**3**
			OH	rhamnoside	**4**

Fig.1. Structures of the isolated compounds from *A. albida*

TABLE 1. Glucose (mg/dl), insulin (µIU/ml), concentrations and insulin resistance of rats after 21 days.

Insulin resistance	Insulin (µIU/ml)	Glucose (mg/dl)	
1.98 ± 0.12	8.96 ± 0.20	89.66 ± 4.92	**Group 1**
4.66 ± 0.21 *	5.05 ± 0.18 *	378.16 ± 25.27 *	**Group 2**
2.06 ± 0.04	8.95 ± 0.34	94.83 ± 1.93	**Group 3**
1.78 ± 0.10	8.31 ± 0.27	94.50 ± 3.66	**Group 4**
3.72 ± 0.35 **	6.78 ± 0.18 **	228.66 ± 20.96 **	**Group 5**
3.17 ± 0.34 **	8.31 ± 0.21 **	155.33 ± 18.06 **	**Group 6**

Data shown as mean ± SE, * significantly different at level $P< 0.05$ relative to control, ** significantly different at level $P< 0.05$ relative to diabetic group.

TABLE 2. Total cholesterol (mg/dl), triglycerides (mg/dl), HDL (mg/dl) concentrations.

LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	
15.52 ± 1.29	57.50 ± 2.07	89 ± 5.74	87.83 ± 3.29	**Group 1**
122.94 ± 5.80 *	38.50 ± 1.83 *	193 ± 2.20 *	196.16 ± 4.02 *	**Group 2**
12.96 ± 0.91	56.50 ± 2.61	94.33 ± 3.33	88.50 ± 3.27	**Group 3**
12.32 ± 0.90	54.33 ± 2.07	96 ± 3.23	86.33 ± 2.37	**Group 4**
39.48 ± 3.52 **	52.50 ± 1.66 **	144.66 ± 2.74 **	120.50 ± 3.26 **	**Group 5**
25.58 ± 2.36 **	55.16 ±2.00 **	113.33 ± 3.48 **	102.33 ± 1.56 **	**Group 6**

Data shown as mean ± SE, * significantly different at level $P< 0.05$ relative to control, ** significantly different at level $P< 0.05$ relative to diabetic group.

TABLE 3. Malondialdehyde (nmol/ml) and Q 10 levels of rats after 21 days.

Q 10 levels	Malondialdehyde(nmol/ml)	
0.71 ± 0.01	199.93 ± 6.94	**Group 1**
0.37 ± 0.01 *	296.51 ± 5.52 *	**Group 2**
0.68 ± 0.01	197.73 ± 7.86	**Group 3**
0.74 ± 0.01	200.56 67 ± 6.59	**Group 4**
0.59 ± 0.01 **	220.53 ± 2.88 **	**Group 5**
0.71 ± 0.02 **	193.26 ± 4.02 **	**Group 6**

Data shown as mean ± SE, * significantly different at $P< 0.05$ relative to control, ** significantly different at $P< 0.05$ relative to diabetic group.

Histopathological Results

Liver

The microscopic examinations of sections of liver of non-diabetic control rats displayed the normal structure of the hepatic lobule. The central vein is encircled by the hepatocytes with eosinophilic cytoplasm and distinct nuclei. The liver sinusoids between the hepatocytes are shown (Fig. 2-A). In addition, the portal areas show normal histological features (Fig. 2-B). Histopathological investigations of the liver of diabetic rats indicated that periportal tract associated with dilated and congested vein. Periportal necrosis and focal necrosis of the hepatocytes that surrounded the portal area are also shown. Some of the nuclei of the hepatocytes were pyknotic (Fig. 2-C). Examination of sections of liver of non-diabetic rats treated with 100 and 200 mg/kg of *A. albida* showed the normal structure of the hepatic lobule (Fig. 2- D, E respectively). Examination of sections of liver of diabetic rat treated with 100 mg/kg of *A. albida* plant showed normal structure (Fig. 2-F), but some heamohrragic areas were seen (Fig. 2-G). On the other hand, histopathological investigations of the liver of diabetic rats treated with 200 mg/kg of *A. albida* plant showed normal structure (Fig. 2-H).

Pancreas

Photomicrographs of rats control pancreas segments showed the exocrine part of the pancreas consisted of tightly packed acini. The interlobular canal, encircledby the supporting tissue. The pancreatic endocrine tissue, Langerhans islets, dispersed across the exocrine tissue (Fig. 3-A). For rat pancreas treated with 100 or 200 mg/kg of *A. albida* extract, the structure of both endocrine and exocrine pancreas appeared more or less as the control (Fig. 3-B, C, respectively). Sections of pancreas of diabetic rats showed atrophy of the cells of the exocrine pancreas, nuclear pyknosis and karyorrhexis, fatty change in the acinus cells, degenerative changes in some endocrine pancreas and interlobular haemorrhage (Fig. 3-D). However, sections of pancreas of diabetic rat and treated with 100 mg/kg of *A. albida* extract showed normal structures of both the endocrine and exocrine pancreas. On the other hand, Intraaciner hemorrhage and some foci of necrosis in the acini were found. Moreover, the examination of the pancreas of diabetic rats treated with 200 mg/kg of *A. albida* showed that the acini of the exocrine pancreas revealed no evident of changes were shown in (Fig. 3-E), and the islets of Langerhans showed enlargement in size and associated with some hemorrhage.
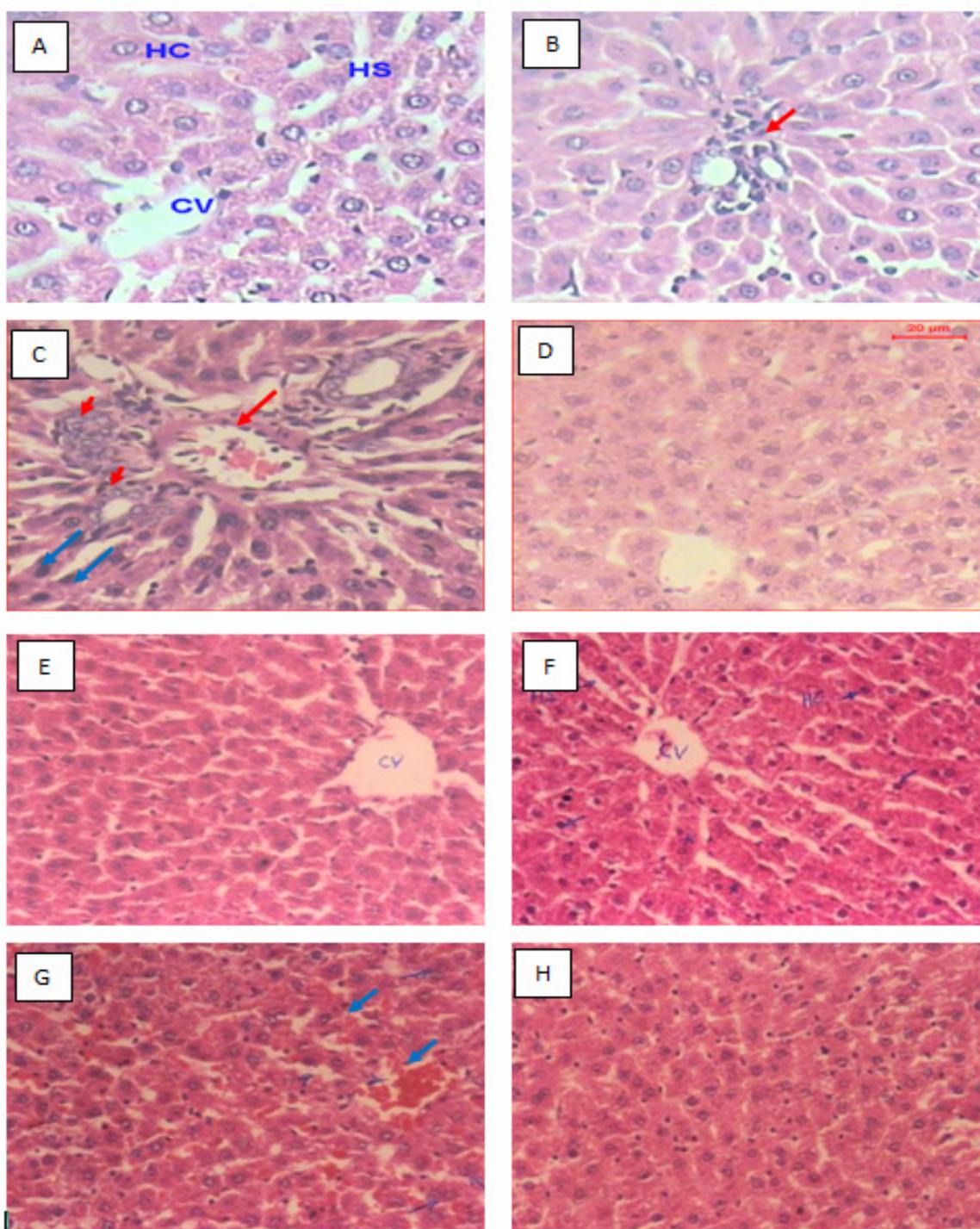
Fig. 2. Sections of liver from A) control of non-diabetic rat shows the normal hepatic lobule structure. The hepatocytes (HC) surround the central vein (CV). The hepatic sinusoids (HS) are shown between the hepatocytes, B) control non-diabetic rat shows the normal portal area (arrow), C) diabetic control rat displays the portal tract with dilated and congested vein (arrow), focal necrosis of the hepatocytes that surrounded the portal area (arrow heads). Some of the nuclei of the hepatocytes are pyknotic (blue arrow), D, E) non-diabetic rats treated with 100 and 200 mg/kg of *A. albida* respectively show the normal structure of the hepatic lobule with a central vein (CV). F) diabetic rats treated with 100 mg/kg of *A. albida* plant shows normal structure, G) some heamohrragic areas were seen (arrows), H) diabetic rats treated with 200 mg/kg of *A. albida* plant shows normal structure (H & E stain X 300).
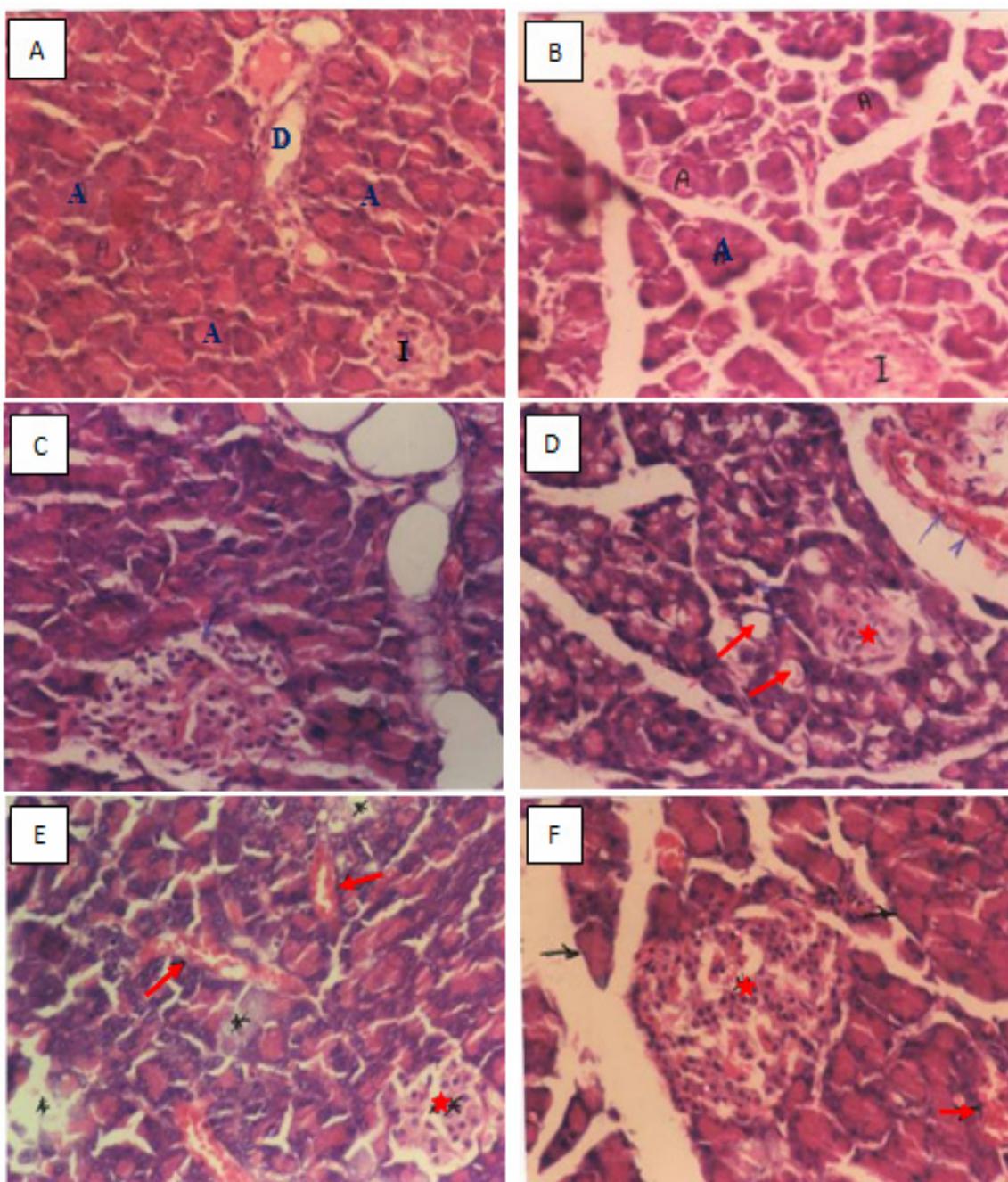
Fig. 3. Sections of pancreas from A) the control rat displays the exocrine parts of the pancreas consisting of tightly packed acini (A). The interlobular duct (D) can be seen encircled by the supporting tissue. The endocrine tissue of the pancreas, Langerhans islets (I), are dispersed across the exocrine tissue, B) non-diabetic rats treated with 100 mg/kg of *Acacia albdai* shows normal structure, C) non-diabetic rats treated with 200 mg/kg of *A. albida* shows normal structure, D) diabetic rat shows the atrophy of exocrine pancreas cells that associated with nuclear pyknosis and karyorrhexis (arrow). Notice the fatty change (arrow) which is characterized by vaculation in the acinus cells. The endocrine pancreas reveals some degenerative changes. Notice the interlobular haemorrhage, E): diabetic rat treated with 100 mg/kg of *A. albida* shows the interaciner hemorrhage. The acini appear more or less like normal except some foci of necrosis. The endocrine pancreas showed normal structure, F) diabetic rat and treated with 200 mg/kg of *A. albida* shows the acini of the exocrine pancreas revealed no evident changes, except some hemorrhage and necrosis (arrow). The islets of Langerhans showed enlargement in size and associated with some hemorrhage (star) (H & E stain X 300).

The presently recognized drug regimens for diabetes mellitus management have certain disadvantages and it is therefore necessary to discover efficient and safe drugs[34]. The current study aims to assess the hypoglycemic, hypolipidemic and antioxidant impacts of *A. albida* plant in STZ-induced diabetic rats. In this research, as expected the STZ administration to rats, led in hyperglycemia, hypoinsulinemia, enhanced total cholesterol, triglycerides, LDL, and malondialdehyde while decreased in HDL and Q 10 levels. The diabetic model used in this experiment was type 2 because some population of pancreatic β-cells were demolished by low dose of STZ (50 mg/kg bw) [34]. Residual β-cells which produced inadequate insulin caused type 2 diabetes[35]. Streptozotocin caused its diabetic effects through selective destruction of pancreatic β-cells [36] and decreasing cell activity lead to lowering the insulin sensitivity for tissue glucose uptake. The increased plasma glucose levels in STZ-induced diabetic rats have been lowered by *A. albida* extract administration. The reduced levels of glucose suggested that *A. albida* extract could have an insulin-like effect on peripheral tissues either by promoting the metabolism of glucose uptake by suppressing hepatic gluconeogenesis [37, 38], or by absorption of glucose through the muscle and adipose tissues[40], by stimulating the process of revitalizing the remaining β-cells[40].

Our study showed that *A. albida* plant extract mainly stimulates the release of insulin. It induced a significant rise of plasma insulin in STZ-treated animals but did not affect insulin in control group. Other effects as rise in triglycerides, total cholesterol and LDL-cholesterol levels and HDL-cholesterol decrease of *A. albida* plant extract in STZ-treated rats might be secondary to a partially restored β-cell function with increased insulin levels. The hypoglycemic effect of *A. albida* plant extract was due to the potentiation of insulin release from the Langerhans islets β-cells. Aseremanthin is a sesquiterpene, β islets may have been stimulated to produce insulin and raise insulin sensitivity to glucose uptake[41]. Lipid profile abnormalities are one of the major complications observed in 40% of diabetic patients[42]. Diabetes brings about a boost in the triglycerides, cholesterol, VLDL and LDL levels[43]. Generally high cholesterol levels and particularly LDL-cholesterol are one of the most common risk factors of coronary disorders. The abnormal high serum lipids concentration in the diabetic cases is mainly due to the increased mobilization of

free fatty acids from the peripheral fat depots, as insulin inhibits the hormone sensitive lipase. Dyslipidemia may be caused by insulin deficiency or insulin resistance, due to the inhibitory effect of insulin on the key rate-limiting enzyme HMG-coA reductase which his responsible for cholesterol-rich LDL metabolism. Initially the deficiency in acute insulin results in increased mobilization of free fatty acid from fat depots which lead to an increase in the generation of cholesterol-rich LDL[44]. The administration of *A. albida* plant extract elevated the serum HDL-cholesterol level and reduced the levels of total cholesterol, LDL-cholesterol and triglycerides. Elevation of LPO is attributed to the enhanced production of reactive oxygen species. In the present study, we observed a MDA formation, the index of lipid peroxidation, was significantly increased in liver of STZ treated animals and that was also manifested by the level of the Q10 which significantly decreased in the serum of STZ treated animals. The plant supplementations potentially reduced MDA level and return the level of Q10 to the normal levels, suggesting that *A. albida* extract might have antioxidant principles to produce such response.

Liver

The histopathology of liver of STZ induced diabetic showed more drastic changes when compared to control group. Damage may be attributed to STZ induced free radical production. The increased production of highly reactive free radicals can deplete GSH store, allowing the reactive intermediate to react with and destroy hepatic cells[45]. When the STZ induced diabetic rats were treated with *A. albida* extract have shown to be protecting the tissue against STZ action. The results obtained reveal that this has more potent antioxidant activity which quenches reactive oxygen species.

Pancreas

Histological results of the pancreatic tissue of the control group showed bluish stained β-cells filling the interior of the islet, while pink stained α-cells are arranged in clusters scattered mainly at the periphery of the islets and also between β-cells. In diabetic group that was injected with STZ, degenerative changes were found mainly in the center of islets reducing the number of the bluish stained β-cells while the pink stained α-cells still present at the islet periphery compared to control group. These findings were in agreement with Al-Khalifa *et al.*, [46], who reported that β-cells numbers significantly decreased in all

diabetic animals relative to the control group. The morphmetric results in *A. albida* treated groups, showed a significant increase in β-cells and islets numbers compared to diabetic rats. While, no significant difference was observed in the islets number relative to the control group. Vessal *et al.*, [47], indicated that plants antioxidants could act on islet functions increase the pancreatic islets numbers and stimulate the release of insulin in diabetic rats.

Conclusion

Exogenous antioxidant store depletion can allow the reactive intermediate to react with and demolish the hepatic and pancreatic cells causing such pathological alterations in the diabetic rats. Actually, the antioxidant enzyme can be raise when treated with *A. albida*, which can improve the oxidative stress, and protects the hepatic and pancreatic tissues of diabetic rats. Thus, these results accentuate that *A. albida* could be a potential agent to attenuate pancreatic oxidative damage and advocate their therapeutic potential for treating diabetes.

Acknowledgment

Conflict of Interests

No conflicts of interest

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التأثيرات المضاده لمرض السكر وزيادة الدهون ومضادات الاكسده للمستخلص الكحولى الميثانولى لنبات الطلح الأبيض فى حالة الاصابه للسكر المستحث بالاستربتوزوتوسين فى الجرذان

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تم فصل سبعة مركبات من نبات الطلح الأبيض؛ تم التعرف عليهم باستخدام الطرق الطيفيه المختلفه وقد أظهرت النتائج المرضية في فئران المجموعة المصابة بمرض السكري وجود احتقان فى المنطقة البابية للكبد والتى كانت مصحوبة بالتهاب خفيف وتليف ملحوظ بالقرب من الوريد المركزي في الكبد. ووجدت زيادة فى الجهد التاكسدى ، والتي عادة ما يتم التخلص منها بواسطة إنزيمات مضادات الأكسدة الداخلية. كما بدا نقص فى مضادات الاكسدة مما سمح للتفاعلات الضارة فى خلايا الكبد والبنكرياس وتدميرها مما تسبب مثل هذه التغيرات المرضية في الفئران المصابة بالسكري. اما فى حالة العلاج بمستخلص الطلح الأبيض, فقد حدث زيادة فى مستوى إنزيم مضادات الأكسدة مما ادى الى تخفيف الجهد التأكسدي وحماية الأنسجة الكبدية والبنكرياسية في الفئران المصابة بالسكري. وبالتالي فإن هذه النتائج تؤكد أنا الطلح الأبيض ممكن أن يكون مخففا من الإصابة واستخدامها كعلاج لمرض السكري.