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Research Article

EVALUATION OF THE HISTOPATHOLOGICAL AND BIOCHEMICAL EFFECT OF *ALOE VERA* AQUEOUS EXTRACT ON DIABETES MELLITUS INDUCED BY STREPTOZOTOCIN IN RATS

ABD EL-KADER AM, AWADALLA EA*, GABR SA, NOUR AH

Department of Zoology, Faculty of Science, Aswan University, Aswan, Egypt. Email: eatemad2000@aswu.edu.eg

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ABSTRACT

Objective: Numerous plants have exciting pharmaceutical activities, but *Aloe vera* (AV) is likely to have many medicinal applications worldwide. Therefore, the present study focused on identifying the potential of AV aqueous extract to protect against complications of diabetes.

Methods: Fifty rats were divided into five groups. The first group, the normal group, received a vehicle solution. The second group, the AV group, received AV aqueous extract (1/2 ml/100 g body weight). The third group was the streptozotocin (STZ)-induced diabetic rat group (i.p. as a single dose), the fourth group was the ameliorative group (a week after STZ injection, rats were given the same daily oral dose of AV extract), and the fifth group was the protective group (rats were treated with the same dose of AV extract for 21 days, and on the 14th day after the start of AV treatment, STZ injection was performed). At the end of the experiment, changes in serum indices, such as insulin, glucose, aspartate transaminase (AST), alanine transaminase (ALT), urea and creatinine, and tissue antioxidants as well as histopathological alterations in the pancreas, liver, and kidney were evaluated.

Results: We found that STZ-treated animals displayed significant increases in tissue lipid peroxidation (LPO, as an indicator of oxidative stress), serum glucose, AST, ALT, urea, and creatinine, with a parallel decrease in the levels of serum insulin and tissue antioxidants. In accordance with these data, several histopathological alterations in the selected organs were observed. When diabetic animals received 1/2 ml/100 g body weight of AV extract, these deleterious effects were ameliorated.

Conclusion: In the current study, AV aqueous extract exhibited an ameliorative and protective effect against the oxidative damage and the associated complications that occurred in different rat organs due to diabetes mellitus induced by STZ.

Keywords: *Aloe vera*, Streptozotocin, Insulin, Antioxidants, Lipid peroxidation.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from defects in the secretion of insulin, the activity of insulin, or both [1]. Streptozotocin (STZ) is a chemical compound that has selective toxicity toward pancreatic beta cells. It is utilized to treat certain cancers of the islets of Langerhans and in medical research to induce type 1 and type 2 diabetes in animal models [2]. Lipid peroxidation (LPO) induced by free radical generation has been related to diabetes and other diseases. Furthermore, STZ-induced diabetes has been confirmed to release free radicals and exhibits most of the diabetic problems, namely, myocardial, cardiovascular, gastrointestinal, nervous, vas deferens, kidney, and urinary bladder dysfunction that are mediated through oxidative stress [3].

The liver is an organ that sustains a severe injury in diabetes, and hepatic impairment is a major complication of diabetes [4,5]. Oxidative stress has been emphasized among the various mechanisms suggested for the pathogenesis of diabetic problems, such as renal and hepatic tissue damage [6-8]. The biochemical changes that occur in diabetes mellitus are exactly like those in liver diseases [9]. The most important indicators associated with liver injury are the levels of plasma alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase, and γ-glutamyltranspeptidase [10,11]. Diabetes mellitus affects renal tissues, leading to diabetic nephropathy, which is a dysfunction of the kidney characterized by specific renal morphological and functional alterations [12].

Available treatments for controlling diabetes and its problems have various side effects with limited benefits; thus, the desire for herbal products with antidiabetic properties and few side effects that are also more cost effective is increasing [13]. The use of herbal medicine over many decades has proven its brilliant future in the treatment of diabetes. Families of plants such as Leguminosae, Liliaceae, Asteraceae, Cucurbitaceae, and many others are commonly used. These plants possess antidiabetic efficacy because they contain polyphenols, alkaloids, coumarins, saponins, fibers, and other phytoconstituents, which significantly decrease blood glucose [14]. The *Aloe vera* (AV) plant (synonym: Aloe barbadensis Miller) belongs to the Liliaceae family, has yellow flowers and grows in dry climates [15], and it has been utilized for medicinal purposes. Most treatment items are produced from AV gel and contain 75 active compounds: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids [16]. In the present investigation, the ameliorative and protective impacts of AV have been assessed in STZ-induced diabetes, with an emphasis on oxidative stress, in rats.

METHODS

Plant collection

The AV leaves used for this investigation were acquired from the research unit at Aswan University for the study of arid land plants. The plant was identified and authenticated by the Department of Botany, Aswan University, Egypt.

Preparation of AV aqueous extract

Fresh succulent leaves of AV were collected. The inner gel component was extracted, homogenized in an electric blender, lyophilized and stored at 4°C [17].

Chemicals
STZ was purchased from Biovision Co. Egypt; a rat insulin enzyme-linked immunosorbent assay kit was purchased from Thermo Scientific Co.; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) assay kits were purchased from Biodiagnostic Co. Egypt.

Experimental animals
Fifty male Wistar rats (weighing 100 ± 20 g) were obtained from the animal house in the Faculty of Science at South Valley University. Animals were housed in well-ventilated clean cages maintained under a 12 h:12 h light: dark cycle at 25 ± 2°C with a relative humidity of 50 ± 5%. Rats were held for approximately 2 weeks before the study began for acclimatization. The animals had access to a pellet diet and water *ad libitum*. Care of the animals and experimental procedures were approved by the Animal Ethical Committee of Aswan University, Egypt, in accordance with the guide for the care and use of laboratory animals.

Experimental design
Fifty rats were grouped into five equal groups comprising ten animals each:
- Group I: Served as the normal group and the rats received the vehicle solution (sodium citrate buffer) only
- Group II: Served as the AV group and rats were treated daily with an oral dose (1/2 ml/100 gmb. w) [18] of aqueous extract of AV
- Group III: Served as the STZ group and rats were treated with a single i.p. injection of 1% STZ at 55 mg/kg b. w. [19]. Animals were permitted to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 1 week, fasting blood glucose levels were measured using an AccuChek glucometer (Manufacturer: Schiffgraben 41, 30175 Hannover, Germany). The rats with blood glucose levels above 250 mg/dl were considered diabetic rats and utilized for the experiment [20]
- Group IV: Served as an ameliorative group, rats were given a single i.p. injection of 1% STZ (55 mg/kg b. w.), and after 1 week, they were given a daily oral dose of AV leaf gel extract (1/2 ml/100 gmb. w.) [18] for 21 successive days
- Group V: Served as a protective group, and rats were given a daily oral dose of AV leaf gel extract (1/2 ml/100 gm b. w.) for 21 successive days. On the 14th day, after the start of AV treatment, they received a single i.p. injection of 1% STZ (55 mg/kg b. w.).

The animals were sacrificed 24 h after the end of the experimental period (21 days).

Blood sampling and serum preparation
After the last dose, animals were sacrificed, blood samples were collected in dried centrifuge tubes, and serum was separated by centrifugation at 3000 rpm for 15 min at room temperature. Serum was collected carefully and kept at −20°C. Concentrations of serum insulin, blood glucose, aspartate transaminase (AST), alanine transaminase (ALT), urea, creatinine, Low-density lipoprotein (LDL)-cholesterol, and triglycerides were assayed according to the manufacturer's instructions.

Estimation of insulin and glucose
Blood samples were collected in heparinized and dried centrifuge tubes, and serum was separated by centrifugation at 3000 rpm for 15 min at room temperature and kept at −20°C for the estimation of serum concentrations of insulin and glucose according to Manley *et al*. [21] and O-toluidine method [22], respectively.

Estimation of serum aspartate transaminase (AST) and alanine transaminase (ALT) concentrations
AST and ALT were estimated at the endpoint by a colorimetric method [23].

Determination of urea and creatinine
Urea was estimated by an enzymatic colorimetric method according to Zawada *et al*. [24]. The creatinine assay is based on the reaction of creatinine with sodium picrate according to Han *et al*. [25].

Estimation of LDL-cholesterol and triglycerides
Cholesterol was estimated by the CHOD-PAP-enzymatic colorimetric method according to Muralikrishna and Nirmala [26]. Triglycerides were estimated by the GPO-PAP-enzymatic colorimetric method according to Hansen *et al*. [27].

Tissue separation
After sacrifice, specimens from the pancreas, liver, and kidney were taken quickly. Samples were then carefully cut into small pieces for further examination.

Biochemical analyses
Tissues were kept frozen at −80°C and then homogenized using a tissue homogenizer Virtiz T-25 Polytronin ice-cold buffer. The homogenates (10% w/v) were prepared using phosphate buffer (pH 7.0) and were centrifuged at 5000 r. p. m. for 1 h at 4°C. The supernatant was used for the following assays:

Lipid peroxidation product (MDA)
MDA was assayed in tissue homogenates according to the method of Ekinci-Akdemir *et al*. [28]. Then, 200 µL of the samples was added to 1 ml of solution (Thiobarbituric acid 25 Mm/L, detergent, and stabilizer). These tubes were covered with glass beads, heated in a boiling water bath for 30 min, cooled and measured at 534 nm against a blank in the presence of a standard.

Superoxide dismutase activity
SOD was assayed in tissue homogenates according to the method of Basu *et al*. [29]. 100 µL of the sample were added to 1 ml of working solution (phosphate buffer + Nitro blue tetrazolium + NADH in a ratio of 10 + 1 + 1 ml) and mixed well. The reaction was initiated by the addition of 0.1 ml of phenazine methosulfate, and the increase in absorbance was measured at 560 nm for 5 min for the control ($A_{control}$) and the sample (A_{sample}) at 25°C.

Catalase activity
CAT was assayed in tissue homogenates according to the method of Sandalio *et al*. [30]. 5 µL of the sample were added to 0.5 ml phosphate buffer, pH7.0, and 0.5 ml H_2O_2. The mixture was incubated for 1 min at 25°C, and a chromogen-inhibitor and peroxidase 4-aminoantipyrine preservatives were added. The solution was then incubated for 10 min at 37°C and measured at 510 nm against a blank in the presence of a standard.

Glutathione peroxidase activity
GPx was assayed in tissue homogenate according to the method of Alam *et al*. [31]. 10 µL of the sample was added to 1 ml buffer, 100 µL NADPH and 100 µL H_2O_2. The solution was mixed well, and then the absorbance was recorded at 340 nm/min over a period of 3 min against deionized water.

Histological studies

Light microscopy

Pieces of the selected organs were collected from all groups, washed in sterile saline and kept in 10% neutral phosphate-buffered formalin (pH 7.0). For microscopic preparations, specimens were dehydrated in a graded ethyl alcohol series (50%–99%), cleared in methyl benzoate and embedded in molten paraffin wax at 58°C–62°C. Tissue sections were prepared at a 5 µm thickness and stained with hematoxylin and eosin [32] for microscopic investigation. Examination of the organ section to assess histological and histopathological alterations was performed under high-power light microscopy (Olympus BX43F, Tokyo 163-0914, Japan). Image analysis was performed using a personal computer, a camera, software (Olympus DP74 Tokyo 163-0914, Japan) and an optical microscope.

Electron microscopy

For ultrastructural examination, the samples were processed, examined, and photographed in the Electron Microscopy Unit at Assiut

University. Small pieces of the selected organs were immediately immersed in a 4F1G/phosphate buffer (pH 7.2) fixation mixture for 3 h at 4°C and then postfixed in 1% OsO_4 (osmium tetroxide) at 4°C for 2 h. The specimens were dehydrated in a graded series of ethanol and then embedded in Epon-araldite mixture in labeled beam capsules. An LKB ultramicrotome was used to obtain ultrathin sections (50 nm thick) that were picked on 200 mesh naked copper grids. Grids were double stained with uranyl acetate for 30 min and lead citrate for 20–30 min. The grids were examined using a TEM 100 CXII electron microscope at 80 KV and photographed using a CCD digital camera Model XR-41.

Statistical analysis

Data are expressed as the mean ± standard deviation The differences between means were tested by one-way analysis of variance followed by the Student's – Newman–Keuls *t*-test using Minitab 12 software. $p < 0.05$ was considered to indicate significance.

RESULTS

Estimation of changes in serum insulin, glucose, AST, ALT, urea, creatinine, LDL-cholesterol, and triglycerides

Table 1 shows that compared with the control group, the AV-group showed nonsignificant ($p > 0.05$) changes in the levels of all estimated serum parameters, while the STZ group showed a significant ($p < 0.05$) increase in the levels of glucose, triglycerides, LDL-cholesterol, AST, ALT, urea, and creatinine and a significant ($p < 0.05$) decrease in the level of insulin. Administration of AV to STZ-treated rats in both the ameliorative and protective groups induced a highly significant ($p < 0.01$) decrease in the levels of glucose, triglycerides, LDL-cholesterol, AST, ALT, urea, and creatinine and induced a highly significant ($p < 0.01$) increase in the serum insulin level.

Estimation of malondialdehyde (MDA) and antioxidant enzyme activity

Information about the effect of AV gel extract on the concentration of MDA and the activity of antioxidant enzymes in the selected tissues of the control and treated groups are presented in Table 2. Again, the AV-group showed non-significant ($p > 0.05$) changes in the MDA concentration and the activity of SOD, CAT, and GPx.

On the other hand, the STZ group exhibited a significant ($p < 0.05$) increase in the MDA concentration and a significant ($p < 0.05$) decrease in the activity of SOD, CAT, and GPx. Administration of AV to STZ-treated rats in both the ameliorative and protective groups induced a highly significant ($p < 0.01$) decrease in MDA and a highly significant ($p < 0.01$) increase in SOD, CAT, and GPx activity.

Histological studies

Pancreas

The effect of AV gel extract treatment on histological changes in the pancreatic tissues of the control and treated groups is shown in Figs. 1 and 2. The pancreas of normal animals (Fig. 1a) and AV-group (Fig. 1b) showed normal pancreatic tissue structure, islets of Langerhans, and surrounding pancreatic acini. In contrast, the pancreas of the STZ group (Fig. 1c) exhibited atrophied islets of Langerhans with degenerated cells of Langerhans. In the ameliorative (Fig. 1d) and protective (Fig. 1e) groups, we observed that the pancreas retained a healthy pattern and that the islets of Langerhans and surrounding pancreatic acini were normal to a large extent.

Beta cells were identified by their characteristic secretory granules in the normal group (Fig. 2a) and AV-group (Fig. 2b). The secretory granules had an electron-dense core of variable electron density (light and dark granules) surrounded by an electron-lucent halo under the limiting membrane. The mitochondria were scattered throughout the cytoplasm. Ultrastructure examination of the beta cells of the STZ-group showed degenerative alterations, which were represented by atrophied nuclei with ruptured nuclear membranes. The mitochondria appeared with electron lucent matrices and disorganization of some Golgi complexes. Moreover, fewer secretory granules were empty and had cytoplasmic vacuoles (Fig. 2c). Administration of AV to STZ-treated

Table 1: Changes in serum insulin, glucose, ALT, AST, urea, creatinine, low-density lipoprotein-cholesterol, and triglycerides

Parameters	Normal group	AV-group	Diabetic group	Ameliorative group	Protective group
Insulin (ng/dl)	2.07±0.82	2.096±0.86#	0.33±0.10*	0.88±0.55**	0.84±0.32**
Glucose (gm/dl)	205.80±1.85	201.68±1.85#	326.40±1.80*	268.50±1.80**	268.80±1.37**
Triglycerides (mg/dl)	5.96±0.44	5.95±0.37#	8.56±2.03*	3.07±0.80**	3.12±1.02**
LDL-C (mg/dl)	62.2±2.33	58.68±2.08#	94.45±2.37*	57.52±2.31**	64.36±2.42**
ALT (U/l)	31.5±1.71	30.74±1.75#	68.2±1.20*	38.10±1.78**	39.20±1.51**
AST (U/l)	58.1±1.71	57.97±1.67#	80.7±1.32*	56.30±1.78**	60.80±1.51**
Urea (mg/dl)	20.44±2.10	18.76±2.97#	61.05±2.17*	28.06±2.67**	30.79±2.37**
Creatinine (mg/dl)	0.34±0.12	0.325±0.13#	2.07±0.82*	0.31±0.55**	0.31±0.33**

Values are means±SD of 10 animals in each group. #Nonsignificant ($p>0.05$) compared to control group. *Significant ($p< 0.05$) compared to control group. **Highly ($p< 0.01$) significant compared to diabetic group. LDL-C: Low-density lipoprotein-cholesterol, AST: Aspartate transaminase, ALT: Alanine transaminase, AV: *Aloe vera*, SD: Standard deviation

Table 2: The effects of *Aloe vera* extract on peroxidation and tissue antioxidants level in streptozotocin-induced diabetic rats

Parameters	Tissue type	Normal group	AV-group	Diabetic group	Ameliorative group	Protective group
MDA	Pancreas	4.36±2.93	3.76±2.86#	10.86±2.90*	4.97±2.20**	4.70±2.76**
	Liver	4.24±2.09	3.96±2.76#	10.56±2.97*	5.82±2.32**	4.87±2.49**
	Kidney	5.60±2.39	4.57±2.86#	11.97±3.90*	4.13±2.16**	4.14±3.04**
SOD	Pancreas	4.68±1.62	5.75±2.56#	4.26±2.23*	7.02±2.39**	6.66±2.31**
	Liver	10.55±2.91	11.76±2.87#	3.55±1.59*	10.97±2.16**	7.84±2.39**
	Kidney	7.52±2.44	7.66±2.86#	4.86±2.90*	7.56±2.55**	6.75±2.52**
CAT	Pancreas	86.90±1.96	90.67±2.87#	49.10±1.42*	74.40±2.08**	74.10±1.38**
	Liver	64.41±1.03	70.57±1.86#	29.00±1.09*	79.60±1.98**	82.00±1.85**
	Kidney	56.20±1.01	58.76±2.97#	46.20±1.77*	100.2±1.83**	74.20±1.10**
GPx	Pancreas	5.68±1.54	5.96±1.57#	5.54±1.34*	7.99±2.20**	7.82±1.88**
	Liver	6.47±1.48	6.85±1.78#	5.45±1.50*	8.15±2.05**	7.77±1.87**
	Kidney	5.46±1.48	5.86±1.67#	5.39±1.42*	7.92±2.19**	7.68±1.80**

Values are means±SD of 10 animals in each group. #Nonsignificant ($p> 0.05$) compared to control group, *Significant ($p< 0.05$) compared to control group, **Highly ($p< 0.01$) significant compared to diabetic group. AV: *Aloe vera*, SD: Standard deviation, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

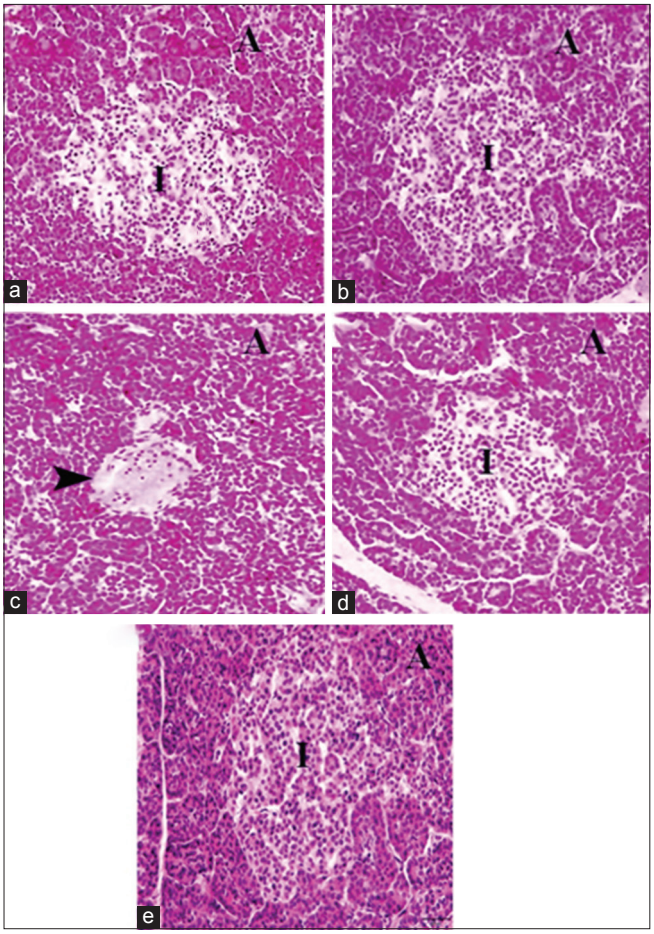

Fig. 1: Pancreatic sections of the control and treated groups. (a and b) Pancreatic sections exhibit closely packed lobules of pancreatic acini (A) around the pale stained Islets of Langerhans (I). (c) Diabetic pancreas section displays pathological changes in the endocrine part of the pancreas represented by degenerated Islets cells (arrowhead). (d and e) Pancreatic sections have a more or less normal appearance of the Islets of Langerhans (I) and pancreatic acini (A). (H and E, ×400 = 50 µm)

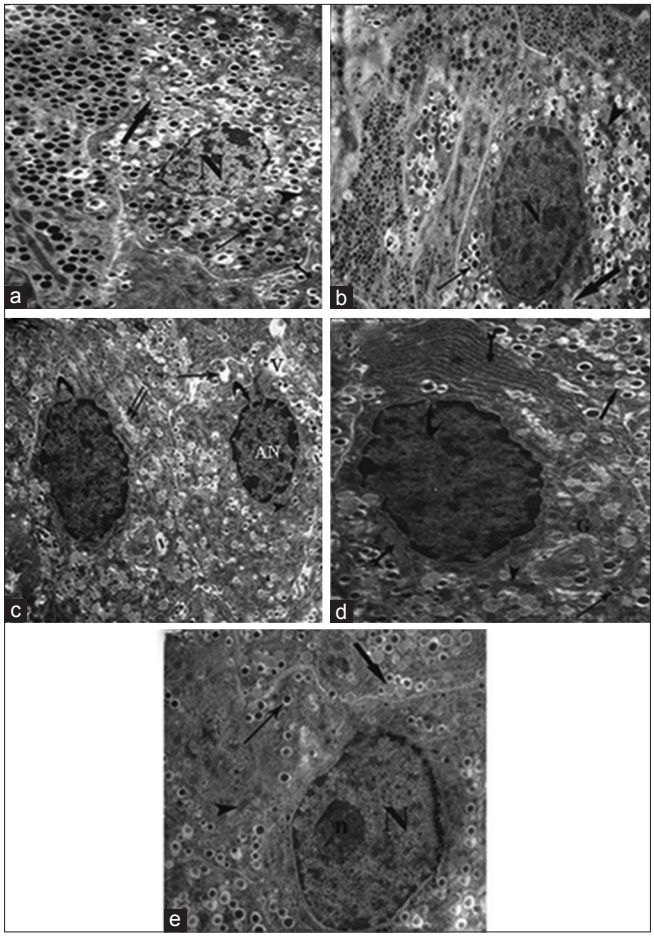

Fig. 2: Electron micrographs of beta cells of control and treated groups. (a and b) Beta cells display euchromatic nuclei (N), the mitochondria (arrowhead) and secretory granules with light (thick arrow) and dark (thin arrow) cores. (c) Beta cell shows atrophied nuclei (AN) with rupture ( ) in nuclear membranes, and mitochondria (arrowhead) with electron lucent matrices and disorganization of some Golgi complexes ( ). Some secretory granules with very wide halos (arrow) and some cytoplasmic vacuoles (V) were noticed. (d) Beta cell reveals a nearly normal structure of rough endoplasmic reticulum ( ), Golgi complexes (G), scattered mitochondria (arrowhead), and secretory granules with light (thick arrow) and dark (thin arrow) cores. Note: Nuclei (N) retained ruptured nuclear membranes ( ). (e) Beta cell shows a more or less normal structure of nucleus (N) with nucleolus (n) and secretory granules with light (thick arrow) and dark (thin arrow) cores. (Original magnification: ×5800)

rats (ameliorative and protective groups) revealed improvements in islet structure, including the disappearance of cytoplasmic vacuoles due to the regeneration of cytoplasmic content and cytoplasmic organelles, such as the rough endoplasmic reticulum, Golgi complex, and mitochondria (Fig. 2d and e, respectively).

Liver

Sections of liver from control animals (Fig. 3a) and AV-treated animals (Fig. 3b) showed normal architecture with normal hepatic cords composed of hepatocytes and healthy central veins as well as blood sinusoids. The majority of hepatocytes displayed eosinophilic cytoplasm with vesicular nuclei. In the STZ group, the liver showed marked pathological changes as evidenced by extensive cell necrosis with pyknotic nuclei. In addition, obvious congestion of the central vein and blood sinusoids is shown in Fig. 3c. STZ-treated rats in both the ameliorative and protective groups that were administered AV showed the good and normal appearance of hepatocytes as well as central veins and blood sinusoids to a large extent (Figs. 3d and e, respectively).

In the electron microscopic analysis, hepatocytes of control (Fig. 4a) and AV-treated animals (Fig. 4b) displayed normal structures and a healthy appearance. Electron micrographs of hepatic cells from the STZ group (Fig. 4c) showed obvious alterations, such as degenerated nuclei with disintegrated chromatin and an irregular envelope. In addition, cytoplasmic vacuoles, many lysosomes, and mitochondria with damaged cristae were noticed. Electron micrographs of hepatocytes from both the ameliorative and protective groups revealed no ultra-pathological impairments compared with those of the diabetic group (Fig. 4d and e, respectively).

Kidney

Sections of normal kidneys (Fig. 5a) and AV-treated animals (Fig. 5b) showed healthy cortex with normal Malpighian corpuscles and renal tubules. Kidney sections from the STZ group showed deteriorated glomeruli in the Malpighian corpuscles and necrotic renal tubule cells with pyknotic nuclei (Fig. 5c). STZ-treated rats in both the ameliorative and protective groups showed nearly normal cellular architecture after AV administration (Fig. 5d and e, respectively).

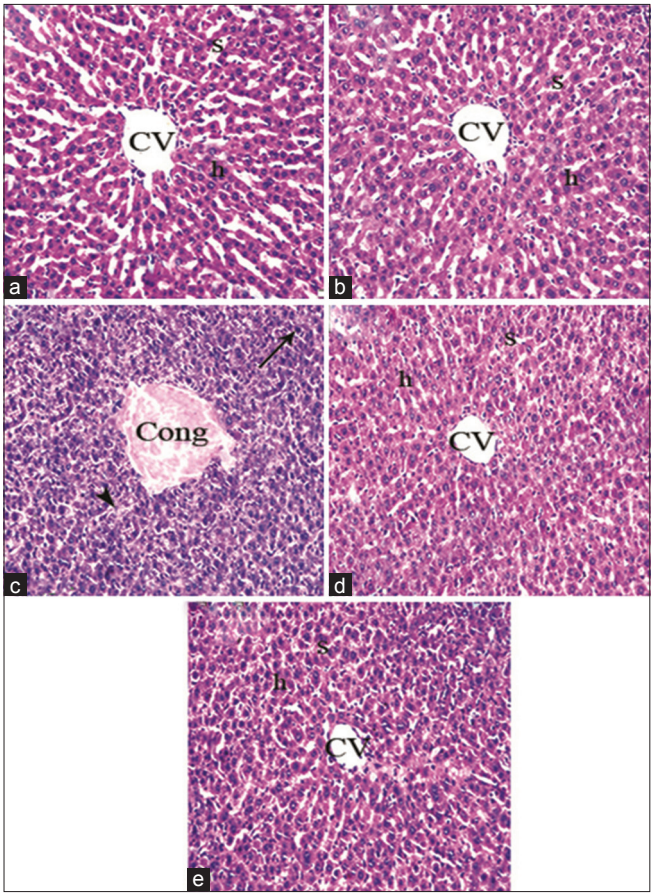

Fig. 3: Liver sections of the control and treated groups. (a and b) Liver sections exhibit normal hepatic cords of hepatocytes (h), central vein (CV) and between the cords of hepatocytes, blood sinusoids (S) are often seen. (c) Diabetic liver section manifests pyknotic nuclei (arrow). Noticeable congestion of the central vein (Cong) and blood sinusoids (arrowhead) were also seen. (d and e) Liver sections show near-normality of cords of hepatocytes (h), blood sinusoids (S), and central vein (CV). (H and E, ×400 = 50 µm)

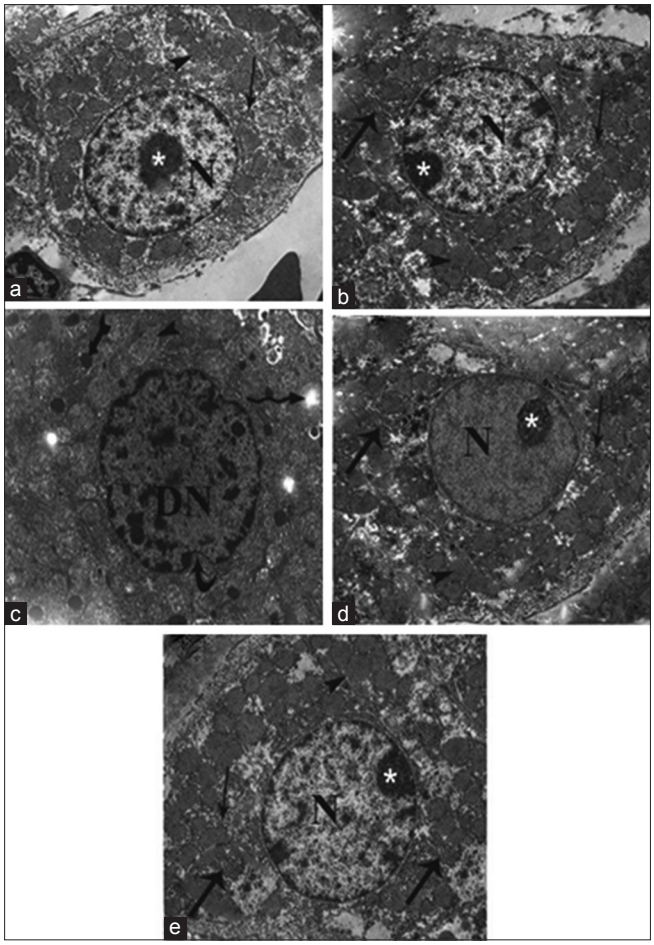

Fig. 4: Electron micrographs of the rat hepatocytes of control and treated groups. (a and b) Hepatocytes reveal normal structure of rounded nuclei (N), nucleoli (star), numerous mitochondria (arrowhead), cisternae of rough endoplasmic reticulum (thick arrow), and glycogen granules (thin arrow). (c) Hepatic cell displays degenerated nuclei (DN) with disintegrated chromatin and irregular envelopes ( ). Cytoplasmic vacuoles ( ), lysosomes ( ), and mitochondria with damaged cristae (arrowhead) were noticed. (d and e): Hepatic cells exhibit more or less similarity to the normal group. (Original magnifications, ×4800)

Electron micrographs from proximal convoluted tubule lining cells of the normal group (Fig. 6a) and AV-treated group (Fig. 6b) exhibited a normal organization of the renal tubular lining cells. The ultrastructural findings of the STZ group showed marked intoxication of the renal tubules with severe degenerative alterations in the nuclei manifested by atrophy of some nuclei and by deformation of the nuclear membrane of other nuclei (Fig. 6c). Again, STZ-treated rats in both the ameliorative and protective groups administered AV showed nearly normal cellular architecture, with relatively normal-appearing basal mitochondria and nucleus with nucleolus. In addition, some electron-dense cytoplasmic bodies were apparent in the cytoplasm of the cell (Fig. 6d and e, respectively).

DISCUSSION

Our data from the current work clearly confirm the antioxidative potential of AV extract in the amelioration of diabetes mellitus-induced oxidative damage in the pancreas, liver, and kidney of rats and changes in other diabetes-related indicators, such as serum insulin, glucose, triglyceride, LDL-cholesterol, AST, ALT, urea, and creatinine. The antidiabetic effects of AV were supported by its antioxidative properties, as evidenced by a decrease in tissue LPO and an increase in cellular antioxidants, such as SOD, CAT, and GPx. Diabetes mellitus is a chronic disease characterized by metabolic disturbances, and its problems include hyperglycemia and hypoinsulinemia [14,33-35]. STZ is widely used in experiments to induce a diabetic model due to its ability to cause β-cell toxicity in mammals [36]. The significant indices in the pathogenesis of diabetes are pancreatic dysfunction and cell death [37-39]. STZ induces diabetes by creating reactive oxygen species and decreasing antioxidant capacity, hence causing cytotoxicity in pancreatic beta cells [40]. The oxidative stress following STZ treatment in the current research is in agreement with previous studies [33, 40]. In addition, our results postulated that the histological results of the pancreas confirmed the ameliorative and protective effect of AV extract in pancreatic tissues. While as in previous studies [41], STZ-treated pancreatic tissues exhibited atrophied islets of Langerhans with degenerated islet cells, AV administration to STZ-treated rats revealed a specific degree of recovery in the cell morphology of the influenced islet cells that closely resembled the ordinary cell morphology of the islet of Langerhans. Interestingly, treatment with AV extract noticeably reduced the STZ-induced increase in serum glucose and lipids as well as tissue LPO but increased the levels of antioxidants and serum insulin. The tissue activities of SOD, CAT, and GPx were also enhanced, suggesting the antioxidative potential of the AV extract. The current study demonstrated the antioxidant effect of AV extract on both normal and diabetic rats. Treatment for 21 days revealed no adverse effects and no significant alterations in the status of tissue LPO, antioxidants, serum

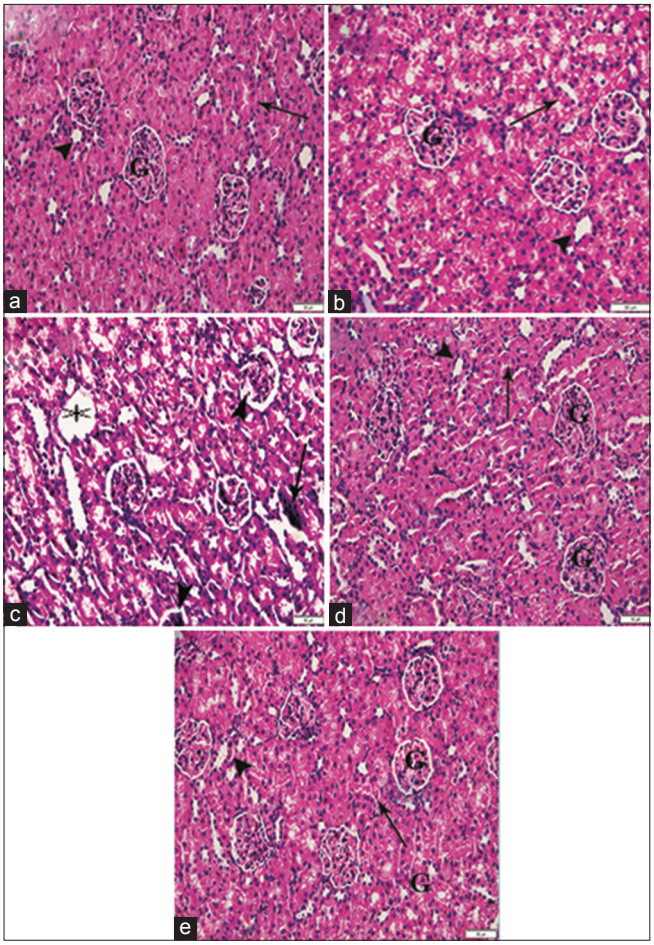

Fig. 5: Kidney sections of the control and treated groups. (a and b) Kidney sections exhibit normal appearance of the Malpighian corpuscle (G), and distal (arrowhead) and proximal (arrow) convoluted tubules. (c) Diabetic kidney section shows necrosis of tubular cells (arrow), atrophy of the glomeruli (arrowhead) and necrotic areas (*). (d and e) Kidney sections display nearly normal Malpighian corpuscle (G), and distal (arrowhead) and proximal convoluted tubules (arrow). (H and E, ×400)

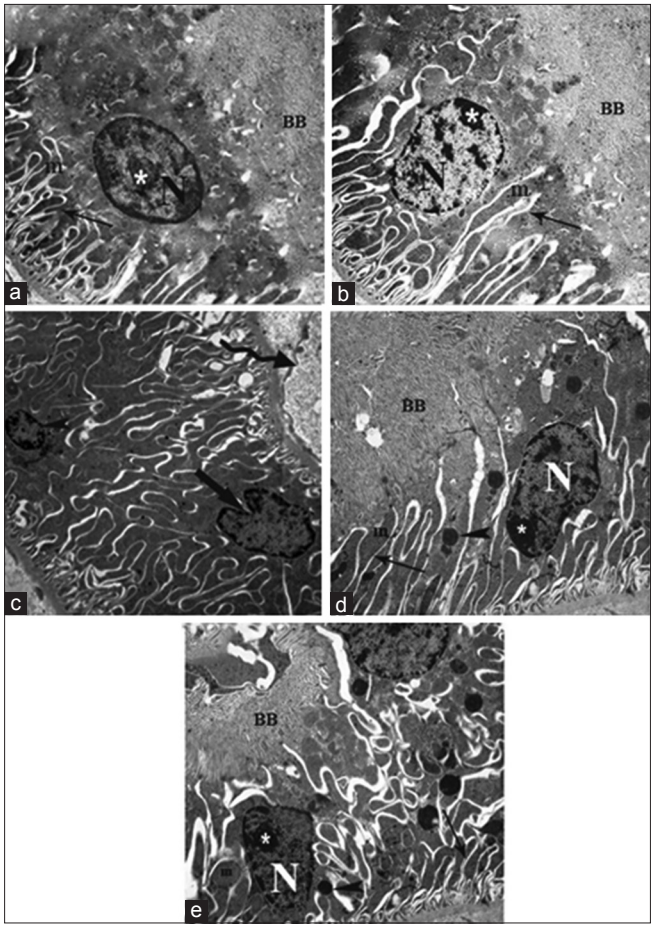

Fig. 6: Electron micrographs of the proximal tubule lining cells of control and treated groups. (a and b) Normal appearance of proximal tubule lining cells with brush borders (BB), nuclei (N), nucleoli (*), mitochondria (m), and infoldings of the basal membrane (arrow). (c) The proximal tubule lining cell shows a ruptured brush border (⇝), abnormally shaped nucleus (thick arrow), and an atrophied nucleus (arrowhead). (d and e) The proximal tubule lining cells reveal a normally shaped nucleus (N), nucleolus (*), mitochondria (m), and infoldings of the basal membrane (arrow) and brush border (BB). Dense cytoplasmic bodies were also observed (arrowhead). (Original magnifications, ×4800)

insulin, glucose, or lipids compared to control animals and displayed positive effects reflected in the diabetic animals with respect to other indices, such as serum glucose, insulin, triglyceride, LDL-cholesterol, AST, ALT, urea, and creatinine.

The recorded increase in serum glucose in diabetic animals may be caused by oxidative stress in the liver [42], where a marked increase in oxidative stress was detected as proved by a significant increase in hepatic LPO. Our findings reinforced those of previous authors, such an antioxidant effect of AV extract on experimental animals [43,44]. In the present study, AV treatment reduced the LPO in all three types of tissues. Moreover, our results are in according with Mohapatra *et al*. [14] and Gabriel *et al*. [43], confirming that the AV-supplemented diet increased SOD, CAT, and GPx activity and decreased MDA activity in plasma. The antioxidant activity of AV extract may be attributed to the opinion of Goda [44], who mentioned that the improvement of antioxidant activities by medicinal herbs is due to their abundant bioactive compounds. In addition, the majority of phenol/polyphenols, alkaloids, enzymes (SOD, CAT, and GPx), vitamins (ascorbic acid), and polysaccharides present in Aloe leaves are known to possess antioxidant activity, with high levels of oxygen free radical scavengers [45]. In addition, our result was similar to the findings of Rajasekaran *et al*. [46] and Nwajo [47] and Abo-Youssef and Messiha [48], who found that AV supplementation reversed the altered oxidative stress parameters, repressing elevated serum MDA levels and increasing blood GSH and SOD levels. Many explanations have been suggested for this antioxidant effect of AV extract. Of these, AV has long been known to have antioxidant potential through repression of free radical formation and enhancement of cellular thiol status [49-52].

Indeed, liver and kidney are represented as major target organs for drugs [53]. In the current work, in addition to pancreatic damage, STZ administration led to hepatic damage causing hepatic malfunction and alterations in the circulating concentrations of hepatic damage enzymes. Monitoring the levels of serum ALT and AST, which are also sensitive serological indicators of hepatotoxicity, are indicative of a clinical diagnosis of the disease and damage to the structural integrity of liver [54,55]. Oxidative stress related to STZ-induced diabetes has been found in response to higher levels of these enzymes in serum [53], as also found in our study, confirming that STZ administration induced liver tissue damage and significant elevation in serum ALT and AST levels. Again, AV administration to animals treated with STZ reduced these two marker enzymes, which was also reinforced by histological examination and revealed a significant improvement in liver histological damage in the STZ-treated group. Similar effects of various other medicinal plants have been observed [39].

The ambient blood glucose level in STZ-treated rats induces a stable increase in the levels of serum urea and creatinine, which are standard markers of renal dysfunction [56-58], as also found in our study. However, these alterations might be due to metabolic disturbances during diabetes by an elevation in the levels of triglycerides and cholesterol. Surprisingly, AV administration to STZ-treated rats in both the ameliorative and protective groups resulted in a reduction in the levels of urea and creatinine compared to those of STZ-treated animals, again suggesting the ameliorative and protective effects of AV extraction kidney tissues. Moreover, the histological pictures of STZ-treated kidneys displayed marked histological changes. The histological evaluation illustrated that AV administration motivated improvement in histopathological changes to near-normal morphology. Thus, AV extract produced a renal-protective effect at the functional and tissue levels. However, AV administration decreased not only glucose but also the levels of cholesterol and triglycerides. This decrease in cholesterol and triglyceride levels by AV administration to STZ-treated rats is also similar to that in earlier reports [59, 60]. As hyperlipidemia has been confirmed to be ameliorated by the administration of antioxidants [61-63], it may be assumed that in our study, AV, being a strong antioxidant, reversed hyperlipidemia through its antioxidative potential. Thus, our study clearly suggests that AV extract has the potential to ameliorate and protect against hyperlipidemia in STZ-induced diabetic rats.

CONCLUSION

Our data obtained from the present study confirmed that all STZ-induced adverse effects were reversed by the simultaneous administration of AV extract through its antioxidative potential. Thus, the AV extract has a protective and ameliorative potential against diabetes mellitus through its antioxidant and antidiabetic potency. In addition, these findings are promising for further clinical examination of the effects of AV extract or ingredients of extraction diabetes mellitus.

AUTHORS' CONTRIBUTIONS

GABR SA designed the study. Awadalla EA and Nour AH carried out the experimental work and drafted the manuscript and they were involved in manuscript editing and finalization. Abd El-Kader AM supervised the work and edited the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

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