

Apple Cider Vinegar (as a Prophetic Medicine Remedy) Exerts Tissue-protective Effects in Streptozotocin-induced Diabetes in Animals

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Abstract Background: Diabetes mellitus (DM) was reported to be related to occurrence of both biochemical and histopathological changes in hepatic tissue. DM-induced tissue damage and decreased immunity may favor carcinogenesis and tumor development in pediatric practice and adults. In this study, we aimed at investigating the hepatoprotective and tissue-protective effects of apple cider vinegar (ACV) against DM-induced hepatic derangements over a relatively long period (30 days). Effects were evaluated using streptozotocin (STZ)-induced DM in rats as an experimental model. **Materials and methods:** DM was experimentally caused by injecting one dose of STZ (65 mg/kg given through intraperitoneal injection). Thirty wistar rats were categorized into three experimental groups: control group, STZ-treated group and STZ plus ACV treated group (2 ml/kg BW). Animals were sacrificed 30 days post treatment. **Results:** Our study revealed that ACV administration induced hepatoprotective effects in diabetic rats. Biochemically, ACV reduced significantly serum glycemic values (glucose, total cholesterol, low density lipoprotein-cholesterol while high density lipoprotein cholesterol significantly increased). STZ-induced DM in rats was associated with marked fatty changes hepatocytes cytoplasm evidenced by accumulation of lipid droplets and inflammatory changes e.g. lymphocytic infiltration. That was confirmed by Electron microscopic evaluation that showed aggregations of polymorphic mitochondria, loss of mitochondrial cristae and condensed mitochondrial matrices. Hepatocytes cytoplasm showed vacuolations with a large number of variable-sized lipid droplets. Hepatocytes rough endoplasmic reticulum got distorted and fragmented into smaller stacks. ACV proved effective in reversing all these metabolic changes. ACV-treated hepatocytes exhibited minimal STZ-induced toxic effects. ACV enhanced recovery of the injured hepatocytes and normalized serum biochemical values. In prophetic medicine, Prophet Muhammad (Allah's apostle peace be upon him) strongly advised eating vinegar and considered it as the best edible as narrated in the prophetic hadith: "vinegar is the best edible." **Conclusion:** ACV is promising as a food additive or as a part of diet in diabetic patients particularly in early stages of DM. ACV slows down DM progress and causes hepatoprotection against the metabolic damages resulting from STZ-induced diabetes mellitus

Keywords: Streptozotocin, diabetes mellitus, apple cider vinegar, liver, rat

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1. Introduction

According to recent estimates, diabetes mellitus (DM) is a growing universal health problem. Over 171 million people have DM worldwide in the year 2000, and the estimated number may increase to 366 million by 2030 [1]. DM is a metabolic disease with a persistently higher than normal blood glucose concentration due to insulin lack (type-1 DM) or deficiency (type-2 DM) [2]. DM-induced tissue damage and decreased immunity may favor carcinogenesis and tumor development in pediatric practice and adults. Currently, DM is a major metabolic disease that is progressively increasing in incidence in all countries constituting a big health concern worldwide. In modern medicine treatments, there is no curative remedy or remedies for treating DM apart from a life-long administration of oral hypoglycemic agents and/or insulin injection. In DM, there exists combined effects of hereditary and environmental factors (diet, life style and sedentary life) ending altogether in deficient insulin production, insulin receptor disturbances or post-receptor abnormalities affecting the metabolism of carbohydrates, proteins and fats in addition to generation of free radicals (reactive oxygen species) causing destructive effects to insulin secreting cells (β -cells of islets of Langerhans of the pancreas), hepatocytes and kidney cells [3].

Management of diabetic patients depends mainly on the dietary and lifestyle factors. Noteworthy, diabetogenic life style habits play an important role in the occurrence of DM etiology, pathogenesis in addition to development of its complications [4]. Liver disease is among the major leading causes of death in diabetic patients (particularly type-2 DM). Mortality rates due to liver disease in diabetic subjects are as high as cardiovascular mortality in diabetic patients. Liver disease in type-2 DM includes fatty liver disease (mostly nonalcoholic), liver fibrosis and cirrhosis and hepatocellular carcinoma [5]. Experimentally, type-1 DM induced in animals using streptozotocin (STZ) or alloxan is a good model for DM induction to simulate the metabolic features observed in patients having uncontrolled DM [6]. STZ-induced DM caused the occurrence of many metabolic changes in different experimental animal models giving a similar metabolic picture to hyperglycemia, non-ketonic DM and tissue changes [7]. Importantly, DM complications are strongly related to impaired metabolism and the metabolic derangements and disturbances in the metabolism of carbohydrates, proteins, electrolytes and lipids (in addition to lack of the anabolic effects of insulin) ending ultimately in diabetic angiopathy, retinopathy, peripheral neuropathy and nephropathy. Minimal reports are there to explain the close relationship between DM complications and liver functions [8].

STZ is a naturally occurring nitrosurea that is commonly used to induce experimental DM in laboratory animals [9]. STZ causes irreversible destruction to pancreatic beta cells of Langerhans (secreting insulin) that results in insulin lack or deficiency [10]. STZ is commonly used to induce DM in laboratory animals as it is toxic to beta cells [11]. Route of administration of STZ is through intravenous or intraperitoneal injection to experimental animals. This causes evident pancreatic insulinitis (inflammation of beta cells) with a final damage

of insulin-secreting beta cells and DM. STZ causes tissue-damaging effects to the pancreas (diabetogenic), the liver (hepatotoxic), the kidneys (nephrotoxic) and also to the stomach (gastric ulceration) [12].

In rats, reported dose of STZ is 65 mg/kg body weight. This dose effectively induced islet damage resulting in hyperglycemia in addition to gastric mucosal ulcerations [12].

Vinegar is an acidic solution gained from apples, cane sugar or artificially from other sources. Apple cider vinegar (ACV) is the best type of vinegar. ACV is a nutritious food additive recommended in prophetic medicine as the best edible. ACV contains potassium, vitamins, minerals and many trace elements [13]. ACV content of potassium enhances serum potassium that is cardioprotective against hypokalemia, enhances soft tissue growth and repair. Moreover, ACV maintains an intact internal body environment via preventing the formation of excessively alkaline urine. ACV breaks down fatty, mucous and phlegm deposits within the body. Metabolically, ACV is a tissue-protective diet acting as a detoxifying and purifying agent that helps elimination and metabolism of many toxic substances that enter the body. ACV exerts hypoglycemic, hypolipidemic effects in addition to antifungal and antibacterial functions [14]. ACV is thought to be beneficial in the treatment of high cholesterol, diabetes and many other diseases [15].

2. Materials and Methods

2.1. Animals

Thirty healthy male Wistar rats (about 180-200 g body weight) were purchased from the animal house facility in Assiut University, Egypt. Animal care, maintenance and experimentation were done according to the guidelines and ethical considerations. Ethical committee approval to achieve the animal experiments was got from the ethical committee of Sohag faculty of Medicine, Sohag University, Egypt. A commercially available balanced diet in addition to tap water ad libitum were provided. Rats included in our study were randomly categorized into three different groups (10 rats each) as follows: Group 1, control rats received no treatment; Group 2, diabetic group (rats administered STZ); Group 3, diabetic rats (received STZ) that were treated with vinegar (2ml/kg body weight diluted with distilled water at a ratio of 1:5) as a sole drinking source). At the end of the experimental period (30 days), rats were sacrificed after overnight fasting.

2.2. Induction of Diabetes Mellitus

Experimental DM was induced by intraperitoneal injection of STZ (Sigma, St. Louis, Mo, USA) at a dose of 65 mg/kg body weight. STZ was dissolved in 0.1 M cold sodium citrat buffer, pH 4.5. After 18 h, animals with fasting blood glucose levels greater than 16.5 mmol/L were considered diabetic and then included in this study [16]. Fasting blood glucose was measured using one touch glucometer (Accu-Chek sensor of Roche Diagnostics, Germany).

2.3. Apple Cider Vinegar

ACV was given to animals in the form of liquid intake (5% concentration) obtained from Faculty of Agriculture, Sohag University, Egypt.

2.4. Biochemical Marker

In this study, we investigated the parameters of serum lipid profile e.g. total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and triacylglycerol (TG). All were estimated using colorimetric enzymatic methods using commercially available kits [17]. Liver function tests were evaluated via measuring the enzyme activities of ALT and AST. Both enzymes were colorimetrically measured as previously reported by Reitman and Frankal [18]. Blood from all rats was collected for biochemical evaluation in two test tubes per rat. Blood tubes underwent centrifugation at 3000 rpm (20 minutes) to get serum that was later kept at -20°C until the suitable time for laboratory evaluation.

2.5. For Light Microscopic Examination

Small sections of the hepatic tissue (using microtome) were immediately fixed for 24 hours in aqueous Bouin's solution and then preserved in 70% alcohol. Specimens water was removed (dehydration). Specimens were immediately cleared in terpineol and embedded in paraffin wax. Sections of $5\mu\text{m}$ thickness were stained with haematoxylin and eosin [19], and then microscopically examined where photomicrographs were made as required.

2.6. For Ultrastructural Evaluation

Liver sections were sliced into smaller cuts. That was followed by fixation in 2.5% glutaraldehyde for 4 hours and also in 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). The samples were post-fixed in a buffered solution of 1% osmium tetroxide at 4°C for one hour. This was followed by dehydration in ascending series of ethyl alcohol, clearing in two changes of propylene oxide, (5 min each), and embedding in Epon-epoxyresin. Semi-thin sections of $1\mu\text{m}$ thickness were cut, picked up on glass slides, stained with toluidine blue and examined for general orientation under a bright field-light microscope. Specimens were then cut again producing ultra-thin sections at 60 nm thicknesses. That was followed by picking up on copper grades. Sections mounted on grids were double stained using uranyl acetate and lead citrate as was previously reported [20].

2.7. Statistical Analysis

Analysis of the data was done using Mean \pm SD using SPSS version 17.0 [21].

3. Results

3.1. Biochemical Results

Blood glucose concentration increased from 142.32 ± 0.45 mg/dl in the control group to 192.13 ± 0.95 mg/dl in the diabetic group rats while in the ACV- treated group, blood glucose decreased to 152.43 ± 1.25 mg (Table 1). In the ACV- treated group (Group III), serum lipid profile was estimated. TC, TT and LDL-c were significantly decreased ($p < 0.05$) HDL-c concentration exhibited significant in the diabetic rats (Table 2) compared to the control group.

3.2. Light Microscopy Observations

Group I (Figure 1): Hepatic sections examined were compared in all groups. Sections of untreated healthy group exhibited the classic picture of portal lobules and hepatocytes characteristics. Normal appearance of portal lobules and hepatic cords was evident. Hepatocytes appeared normal in morphology and distribution among which blood sinusoids existed. Hepatocytes were normal and radiating from a central vein. Liver cords were lined with endothelial cells with the macrophages von K upffer cells. Hepatic lobules were isolated from each other by loose connective tissues containing the portal triads (tributaries of the portal vein, branches of the hepatic artery and narrow bile ductules).

Group II (Figure 2 and Figure 3): The liver of STZ- treated rats exhibited dilatation and congestion of the central veins. Dilated congested central vein was irregular in outline and appeared composed of damaged and separated endothelial cells. Intact and hemolyzed blood cells filled the massively dilated central vein in addition to the presence of an inflammatory infiltrate in the portal tract. The inflammatory infiltrate was variable in intensity from one place to another inside the portal tracts. K upffer cells were actively proliferating markedly. Kupffer cells increased in size and number and location inside the lumina of sinusoids. Fatty changes were observed in hepatocytes.

Group III (Figure 4): The histological structure of the liver of most diabetic rats treated with ACV revealed little pathological change when compared with diabetic rats only and partly restored their normal configuration. The hepatocytes were more or less normal in morphology, well-organized while cytoplasmic vacuolations disappeared. Hepatocytes nuclei were normal in shape having a spherical outline that was centrally located apart from few pyknotic ones.

Table 1. Mean \pm standard deviation and ranges of blood glucose levels in the three groups (mg/dl)

Group	Levels of glucose mg/dl
Group I (Control)	142.32 ± 0.45
Group II (Diabetic)	$192.13 \pm 0.95^*$
Group III (treated with ACV)	$152.43 \pm 1.25^*$

* $P \leq 0.05$.

Table 2. Mean \pm standard deviation and ranges of lipid levels in the three groups (mg/dl)

Group	Total Cholesterol (TC) mg/dl	Total Triacylglycerol (TT) mg/dl	HDL Cholesterol (HDL-c) mg/dl	LDL Cholesterol mg/dl
Group I (Control)	205.26 ± 0.85	39.38 ± 6.44	43.42 ± 4.51	144.52 ± 6.22
Group II (Diabetic)	262.43 ± 1.37	61.12 ± 2.61	36.11 ± 1.32	215.11 ± 1.04
Group III (treated with ACV)	$219.4 \pm 2.3d$	54.53 ± 2.11	44.52 ± 12.6	164.12 ± 1.4

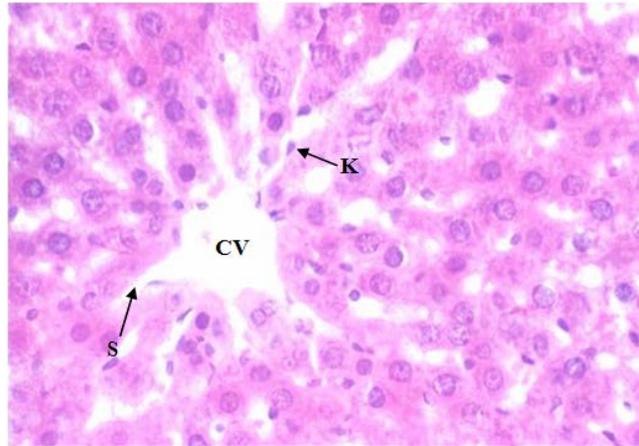


Figure 1. Photomicrograph of liver section of the control group showing normal hepatic cords radiating from a central vein (CV), blood sinusoids (S) and Kupffer cells (K). (X. 400)

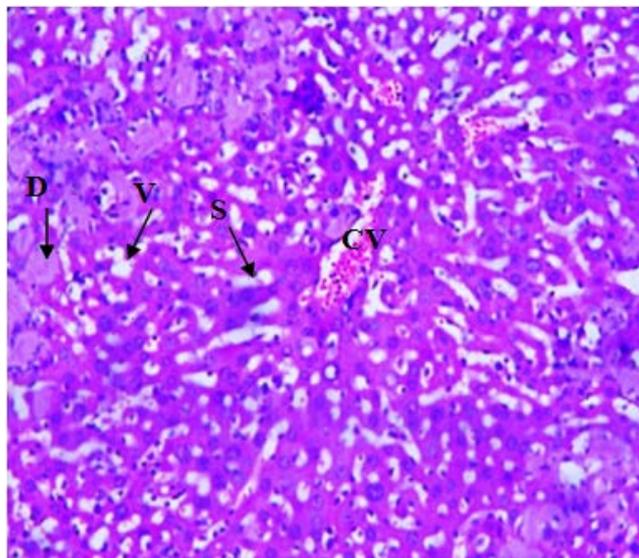


Figure 2. Photomicrographs of liver sections of diabetic rats after treatment with streptozotocin. Liver sections showing marked fatty change (V) of most of the hepatocytes, deteriorated nuclei of the hepatocytes and necrotic hepatocytes (areas of degeneration D) with loss of the regular arrangement of hepatic configuration and dilatation of some hepatic sinusoids (S). Dilated congested central vein (CV) with apparent erosion of its endothelial lining, besides its dilatation together with Kupffer cells (K) proliferation in between the hepatocytes and lymphocytic infiltration (L)

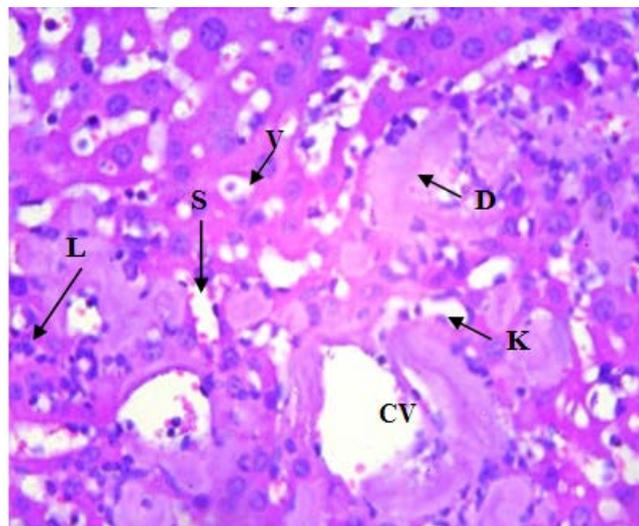


Figure 3. Photomicrographs of liver sections of diabetic rats after treatment with streptozotocin. Liver sections showing marked fatty change (V) of most of the hepatocytes, deteriorated nuclei of the hepatocytes and necrotic hepatocytes (areas of degeneration D) with loss of the regular arrangement of hepatic configuration and dilatation of some hepatic sinusoids (S). Dilated congested central vein (CV) with apparent erosion of its endothelial lining, besides its dilatation together with Kupffer cells (K) proliferation in between the hepatocytes and lymphocytic infiltration (L)

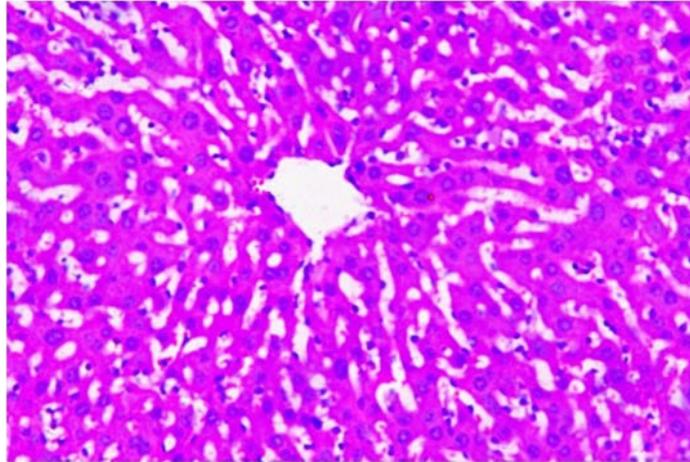


Figure 4. Photomicrograph of liver section of a diabetic rat treated daily with ACV showing that the hepatocytes partly restored their normal configuration (X. 200)

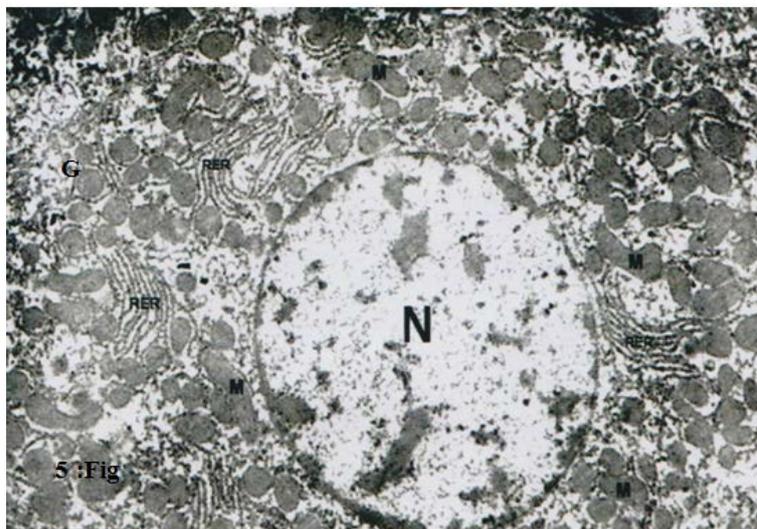


Figure 5. Electron micrograph of liver section of a control rat showing that: The cytoplasm of hepatocyte is occupied by rough endoplasmic reticulum (RER), mitochondria (M), glycogen deposits (G) and the nucleus (N). (X. 4000)

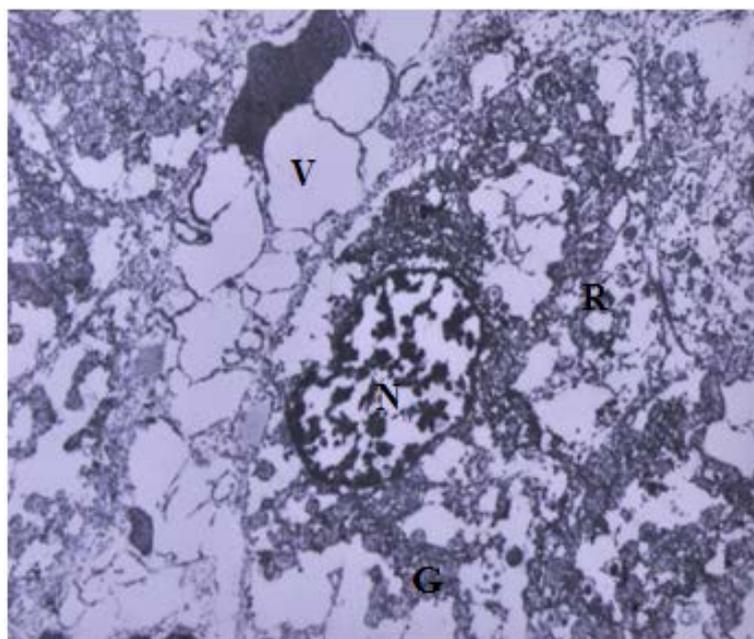


Figure 6. Electron micrographs of liver sections of diabetic rats showing hepatocyte with swollen mitochondria (M), most of them have lost their cristae, the nucleus (N) with irregular nuclear envelope, presence of large lipid droplets (V) and fragmented rough endoplasmic reticulum (RER). (X.3500)

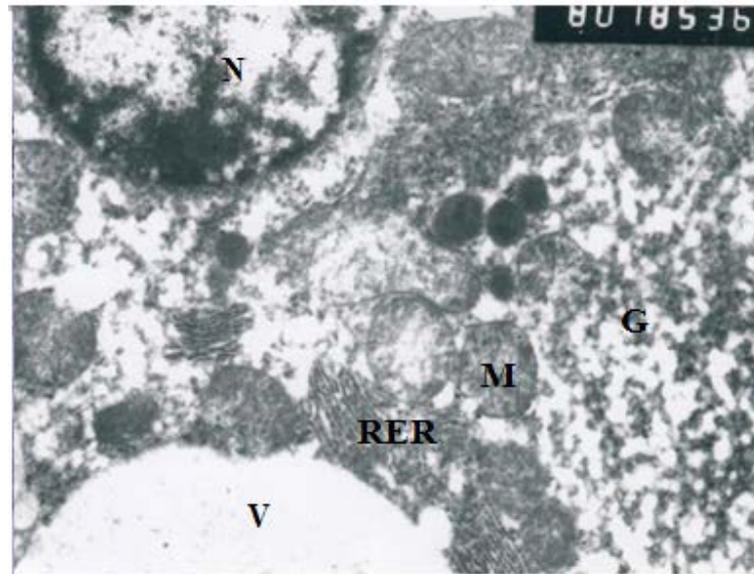


Figure 7. Electron micrographs of liver sections of diabetic rats showing hepatocyte with swollen mitochondria (M), most of them have lost their cristae, the nucleus (N) with irregular nuclear envelope, presence of large lipid droplets (V) and fragmented rough endoplasmic reticulum (RER). (X.3500)

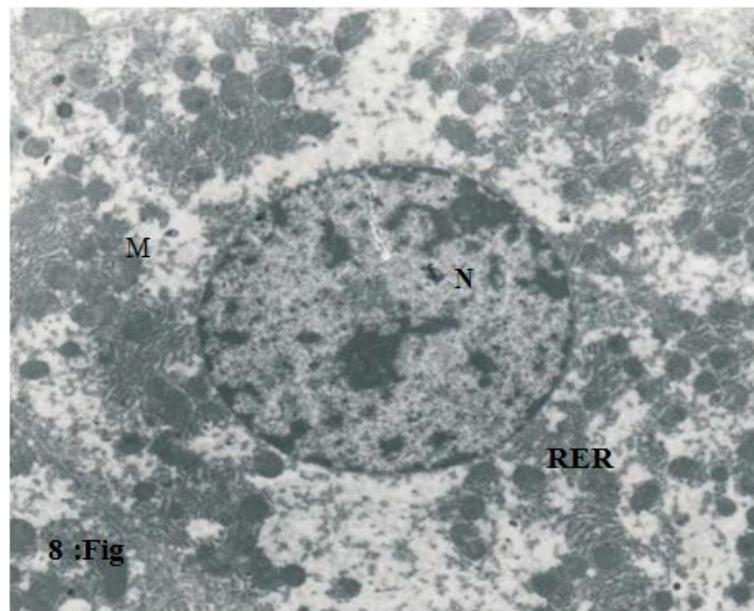


Figure 8. Electron micrograph of liver section of diabetic rats treated daily with ACV showing a hepatocyte with mitochondria (M) in the form of rounded configuration, rough endoplasmic reticulum (RER) in the form of parallel cisternal localization near the nuclear envelope are scattered into the cytoplasm. The nucleus (N) with distinct regular nuclear envelope and nucleoplasm with euchromatin and heterochromatin (X. 3600)

3.3. Ultrastructural Observations

Group I (Figure 5): The ultrastructure of the liver of the control rat is shown in Figure 5. The cytoplasmic organelles as well as the nuclei of the hepatocytes exhibited the normal ultrastructural appearance. The cytoplasm contained many mitochondria scattered.

In many parts inside the cytoplasm. Mitochondria morphology was spherical to ovoid in outline with well-formed and regularly-organized cristae. Hepatocytes RER appeared as closely packed parallel flattened cisternae studded with ribosomes. Significant electron-dense glycogen rosettes or granules were present and marked. Electron microscopic images of hepatocytes nuclei were spherical in outline with a distinct nuclear envelope. Ultrastructure of nucleoplasm showed the presence of many aggregations of different heterochromatin and euchromatin.

Group II (Figure 6 & Figure 7): The electron micrographs of the liver cells of rats treated with STZ revealed marked cytopathological alterations in hepatocytes mitochondria. Liver cells mitochondria exhibited inflammatory changes e.g. swelling, marked aggregation of mitochondrial materials causing loss of mitochondrial cristae. There was abundant rough endoplasmic reticulum that was usually localized near the mitochondria. Also, cisternae of the rough endoplasmic reticulum appeared damaged and broken into smaller stacks.

Group III (Figure 8): Ultrastructural examination of the hepatic sections of investigated rats revealed marked recovery (partial and total) of the cytoplasmic organelles (after ACV treatment). Hepatocytes mitochondria were normal in appearance to a large extent. Hepatocytes exhibited well-developed rough endoplasmic reticulum evident as parallel and flattened cisternae filled with ribosomes with a little amount of lipid droplets.

4. Discussion

DM is well-known for its hyperglycemic effects but little was reported about associated pathological pictures in different body tissues particularly when speaking about drug-induced DM. Simple economic nutritional supplements e.g. vinegar may be excellent dietary additives to decrease glycemic levels. Our study carries a lot of hope in introducing a beneficial, cheap and natural nutritional treatment to diabetic subjects. ACV is not only a hypoglycemic in diabetic mice but also a tissue-protective that guards against hepatocarcinogenesis that is usually based on inflammation, cellular damage and impaired immunity as present in DM. ACV carries a lot of hope in reducing the risk diabetes due to its antihyperglycemic effect in diabetic rats. Treatment with experimental DM (induced by STZ) with ACV caused a significant drop in serum glucose level compared to the diabetic group. This could be attributed to the existence of many active antioxidant ingredients in ACV e.g. acetic acid and organic acids that are tissue protective to the pancreas facilitating the secretion of insulin from beta cells of islets of Langerhans. ACV-induced antidiabetic effects are in part due to delaying the rate of gastric emptying, slowing the rate of sugars and carbohydrate absorption in addition to improving satiety [22] while acetic acid increases the entry of glucose into the tissues leading to normalization of serum glucose and better control of glycemia [23]. The mechanisms beyond ACV-induced hypoglycemia are not well-known. Acetic acid content of vinegar may impede the digestion of starch molecules causing decreased post-prandial glucose absorption [24]. Similar reports proved that ACV ingestion delayed hyperglycemia occurring after a carbohydrate-rich vinegar breakfast [25]. Precise effects of ACV upon insulin action in peripheral tissues e.g. skeletal muscles and adipocytes needs further research studies.

Our article was pioneering also in reporting evident significant decreases in many biochemical parameters e.g. serum LDL-c, TC, TT concentrations significantly dropped in ACV-treated group. This may be attributed to the fact that acetic acid (active component in vinegar) causes a significant lowering in levels of serum cholesterol through suppressing hepatic lipogenesis via enhancing fecal bile acid elimination [24]. It is well-known that acetic acid produces acetate *in vitro* that is later metabolized by tissues activating AMPK pathway to enhance lipid homeostasis and explain the hypolipidemic effects of ingested acetic acid in animals [27]. ACV induced a significant decrease in serum HDL-c concentration that was markedly increased in the diabetic group. In addition, serum TC got significantly decreased upon administration of 0.3% (w/w) acetic acid over 19 days routine diet having 1% cholesterol [23]. This is in agreement with previous reports where vinegar induced hypolipidemic effects in experimental animals [28]. ACV proved effective in controlling the serum lipid profile in all groups (healthy and diabetic rats) via suppressing serum LDL and TG while increasing serum HDL [29]. In the present study, marked histological and ultrastructural alterations were observed in the liver of rats treated with STZ. The histological changes included disorganized hepatic cords, fatty changes in the cytoplasm of the hepatocytes and mononuclear leucocytes, inflammatory

cells infiltration, as well as diffuse proliferation of Kupffer cells. Similar observations have been reported previously in the liver of rats and rabbits treated with STZ where there was erosion of the endothelial lining cells of the hepatic sinusoids with activation of the phagocytic Kupffer cells [30]. Similar observations were also obtained in the hepatic tissue of animals treated with alloxan [30], in the liver of hyperlipidemic rats [32] and in the pancreas of rats treated with STZ [33]. These results are in agreement with previous reports.

In the present study, the ultrastructural alterations were observed included, aggregation of opaque mitochondria, hepatocyte necrosis and hypertrophy of the rough endoplasmic reticulum. Many lysosomes, lipid droplets and active Kupffer cells were also noticed. Devastation of mitochondria was displayed; manifested obvious hypertrophy or swelling and condensation of their matrices. Similar mitochondrial injuries were obtained in the hepatocytes of hyperlipidemic rats. The mitochondria were totally damaged as evidenced by losing their internal ridges, cristae and matrices. Also the present results showed that the cisternae of RER were fragmented into smaller stacks in the liver of rats treated with STZ, which are in accordance with previous reports [32] where distinct changes in the endoplasmic reticulum of the hepatocytes after treatment with diclofenac. ACV is commonly used as a traditional edible in Arabic societies. In prophetic medicine, ACV is a highly recommended food additive where it was narrated that prophet Muhammad peace upon him considered vinegar as the best edible and recommended eating it in the famous hadith: "vinegar is the best edible [35]".

Our data are in agreement with previous reports where ACV was quite tissue-protective against oxidant-induced injury and lipid peroxidation to erythrocytes, nephrons, and hepatocytes. ACV decreased the serum lipid levels in mice fed with high cholesterol diets. Interestingly, ACV proved to possess antioxidant effects through ROS scavenging. ACV inhibits lipid peroxidation and malondialdehyde formation [35].

Moreover, the therapeutic benefits of ACV on normalizing the glycemic response were evident in our study possibly due to the presence of many organic acid contents in vinegar, mainly acetic acid. Importantly, fatty acids in ACV e.g. acetic and propionic acids were reported to sensitize tissues to glucose and insulin effects (when vinegar was used as an edible added to white bread in healthy subjects). Moreover, addition of ACV to a carbohydrate-rich diet was reported to normalize the glycemic control in type-2 DM subjects. The potential mechanisms of action proposed for acetic acid to decrease blood glucose (after meals) and insulin responses are thought to be due to delaying stomach evacuation. Thus, inclusion of ACV ingestion causes a significant decrease in serum glucose that usually rises maximally after meals [36].

5. Conclusion

ACV has potential benefits in diabetic rats though decreasing blood glucose, LDL-c and total cholesterol concentrations. Moreover, it caused increase of HDL-c. Using ACV may have a beneficial effect as a nutritional therapy in diabetic patients.

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