



# **Hyperbaric Oxygen Therapy Ameliorates the Harmful Effects of Dexamethasone Induced Diabetes on Liver and Pancreas of Adult Male Albino Rats**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author NFE designed and performed the experiments, collected the data, performed the statistical analysis, did the entire write up of the manuscript, wrote the protocol and wrote the first draft of the manuscript. Author AAA managed the literature searches and proof read the study. Author MEE designed the study, helped during all steps, managed the analysis, revised all details of the study and managed the literature searches and proof read the study. Author SAE managed the literature searches and proof read the study. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** This study was done to clarify the effects of hyperbaric oxygen therapy on the harmful changes of liver and pancreas of adult male albino rats after dexamethasone induced diabetes.

**Place of Study:** Faculty of medicine, Tanta University, Egypt.

**Methodology:** Thirty two adult male albino rats were divided into; group I (control), group II (Hyperbaric oxygen treated), Group III (induced diabetes - non treated group) and Group IV (induced diabetes + Hyperbaric oxygen treated group). Induction of diabetes was done by dexamethasone injection for ten days. Hyperbaric oxygen was given once per day for 5 sessions in group II and IV. The rats of all groups were sacrificed after the 20<sup>th</sup> day. Specimens of liver and pancreas were subjected to microscopic examination.

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**Results:** Rats from group III showed a highly significant increase of blood glucose compared to the controls. Treated rats in group IV showed highly significant decrease in blood glucose compared to group III. Hepatic steatosis and histopathological changes of pancreatic acini and islets of Langerhans were noticed in untreated diabetic rats. Group IV after Hyperbaric oxygen therapy revealed partial improvement of histological and ultrastructural effects of diabetes on the liver and pancreas.

**Conclusions:** HBOT is partially effective in reducing blood glucose level and improving the hazardous effect of untreated diabetes on the histological and ultrastructural architecture of the liver and pancreas of male albino rats.

*Keywords: Hyperbaric oxygen therapy; dexamethasone; diabetes; liver; pancreas.*

## 1. INTRODUCTION

Hyperbaric oxygen therapy (HBOT) is a new method of non-invasive medical treatment that was widely used in the last few years. It is breathing 100% oxygen at a pressure above the atmospheric pressure at sea level (>1 atmosphere absolute (ATA), 760 mmHg) [1,2]. It is used in treatment of burns [3], femoral neck necrosis [4], acute ischemic stroke [5] and other medical conditions. HBOT was found also to have great benefit in cases of decompression sickness, carbon monoxide poisoning, arterial gas embolism and improving hyperglycemia in type 2 diabetes [6]. Many authors proved that, it is effective in treatment of idiopathic sudden sensorineural hearing loss [7] and obesity in cases of metabolic syndrome [8].

It has been suggested that during HBOT, the plasma becomes saturated with oxygen to a level that improves systemic or local hypoxia and support tissues with minimal need for extracting the oxygen carried by haemoglobin. This leads to a number of beneficial biochemical, cellular, and physiological effects [9]. HBOT was found to be effective in management of chronic wounds especially in diabetic foot ulcers through enhancing vascularization and improving the antibacterial activity [10].

The patient is placed in a specially designed chamber at which the high pressure (compression) is created artificially. This chamber may be a monoplace (single person) or multiplace (2 to 14 patients) chamber. Monoplace chambers are compressed with pure O<sub>2</sub>. However, multiplace chambers are compressed with air and patients breathe pure O<sub>2</sub> through a tight face mask. The arterial O<sub>2</sub> tension increases up to 2000 mmHg while O<sub>2</sub> levels in tissues reach 200 to 400 mmHg during treatment [11].

Diabetes is a disease that affects the body ability to produce or use insulin leading to

hyperglycemia. It is a worldwide problem that is considered the major epidemic disease of this century. It is estimated that 7.7% of adults in developed countries have type 2 diabetes mellitus (T2DM). By 2030, it is predicted that the number of diabetic patients will increase by 20% in developed countries and by 70% in developing countries. It is a major risk factor for cardiovascular diseases with up to 10 times increased risk of cardiovascular events compared to age matched non diabetic individuals [12].

Diabetes is associated with many other complications. Persistent hyperglycemia induces reactive oxygen species production that leads to poor wound healing. It is known that diabetes causes multi-organ failure including pancreatic  $\beta$ -cell damage, retinopathy, neuropathy, nephropathy, atherosclerosis and myocardial infarction [13].

Dexamethasone is a long acting synthetic glucocorticoid that is widely used in inflammatory and allergic conditions [14]. Despite its therapeutic uses, various side effects are associated with its overconsumption [15]. One of these side effects is hyperglycemia and insulin resistance. Thus, it has been used in experimental work for production of an animal model of diabetes [16]. The aim of this study was to evaluate the effects of hyperbaric oxygen therapy on the harmful changes in liver and pancreas of adult male albino rats after dexamethazone induced diabetes.

## 2. MATERIALS AND METHODS

### 2.1 Animals

In this study, thirty two (32) adult male albino rats of average weight (180-220 grams each) were obtained from the animal house of faculty of medicine, Tanta University, Egypt. The rats were housed in clean properly ventilated cages under

similar environmental conditions and fed on the standard laboratory diet ad libitum.

## 2.2 Experimental Design

The rats were divided into four groups (8 rats each) as follow:

### 2.2.1 Group I (Control)

The animals of this group were held without any medication and sacrificed after twenty days from the onset of the experiment.

### 2.2.2 Group II (HBO treated non diabetic group)

The animals of this group were held without any medication for ten days then were exposed to HBOT of once per day on the 11<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup>, 17<sup>th</sup> and 20<sup>th</sup> day from the onset of the experiment then sacrificed.

### 2.2.3 Group III (Induced diabetes - non treated group)

Each animal received Dexamethasone by intraperitoneal (IP) injection in a dose of 10 mg per kg body weight for ten days for induction of diabetes [16]. Proved diabetic rats were held without any medication for 10 days after induction of diabetes then sacrificed on the 20<sup>th</sup> day of the experiment.

### 2.2.4 Group IV (Induced diabetes + HBO treated group)

Diabetes was induced by IP Dexamethasone injection in the same dose used in group III. Then, proved diabetic rats were given HBOT once per day on the 11<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup>, 17<sup>th</sup> and 20<sup>th</sup> day from the onset of the experiment.

## 2.3 Chemicals

Dexamethasone ampoules were purchased from Amriya for pharmaceutical industries, Alexandria, Egypt. Each ampoule contains 8 mg<sup>2</sup>/ml dexamethasone.

### 2.3.1 Hyperbaric oxygen therapy

HBOT was given at Alexandria Vascular and Diabetic foot Center (AVC), Smouha, Alexandria, Egypt. HBO of 2.4 ATA was given for 60 minutes /day 5 times over a period of 10 days starting 10 days from the onset of the experiment. HBO was administered to the rats using a locally

manufactured hyperbaric chamber [17,18]. This small animal chamber was connected to the Hipertech multiplace double compartment hyperbaric chamber used in AVC. Each HBO session included 5 minutes for compression and 5 minutes for decompression.

### 2.3.2 Hipertech multiplace hyperbaric chamber (Fig. 1A & B)

It is a double compartment multiplace hyperbaric Oxygen chamber. It consists of two rooms; one of these rooms contains eight chairs at which eight patients can receive HBOT at the same time. The other room is isolated and contains two chairs A1 & A2. The pressure in the chamber and amount of Oxygen given is controlled by a control unit that is present outside the chamber.

### 2.3.3 The locally manufactured hyperbaric chamber (Fig. 1C & D)

This small animal chamber was made of glass to provide a closed unit for the rats. It was designed to contain 8 rats [17,18]. Two openings were made at its cover to allow two tubes to pass through them. One of these tubes delivered HBOT to the rats of 2.4 ATA, while the other tube provided outlet of the expired air from the rats. The animal chamber was put in the isolated room of HBO chamber.

## 2.4 Body Weight Measurements

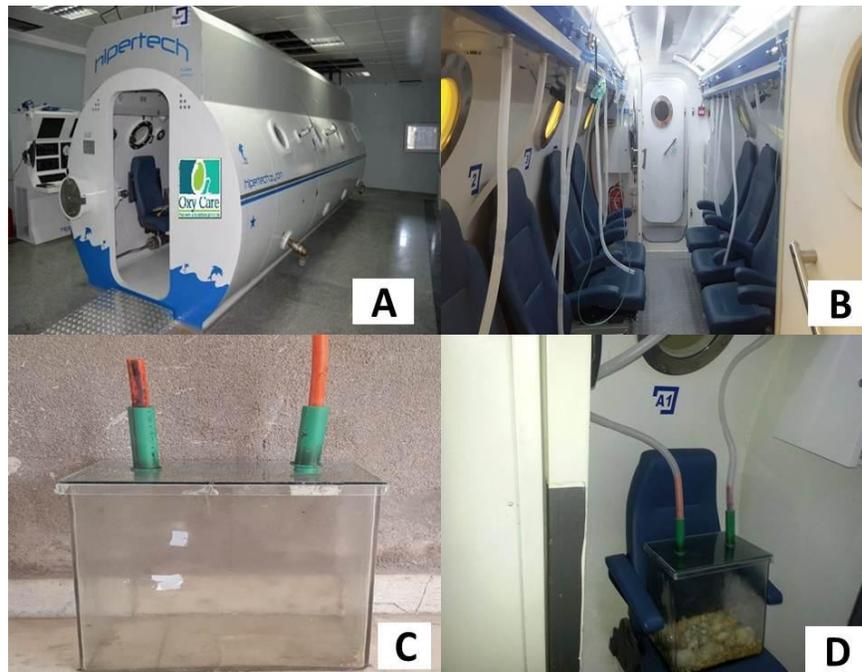
All rats were weighed 3 times; at the onset of the experiment, on the 10<sup>th</sup> day and on the 20<sup>th</sup> day before dissection. Then all rats were anaesthetized with suitable amount of chloral hydrate and sacrificed.

## 2.5 Blood Glucose measurements

Blood glucose level was estimated by using a glucometer used for home diagnostics (One Touch Ultra 2 - Blood glucose meter, manufactured for LifeScan Europe 6300Zug, Switzerland). A drop of blood was obtained from the tail tip in the early morning [19]. The blood glucose was checked 3 times for each rat; at the onset of the experiment, on the 10<sup>th</sup> day and on the 20<sup>th</sup> day one hour after the last HBO session.

## 2.6 Sample Preparation and Examination

After dissection, Liver and pancreas of the rats were extracted and each was divided into



**Fig. 1. The hipertech multiplace double compartment hyperbaric chamber used in AVC (A). The eight chairs room of the multiplace HBO chamber (B). The locally manufactured HBO chamber for small animals (C).The small animal chamber inside the isolated room of HBO chamber during the session (D)**

two halves. One half was fixed in 10% formal saline for light microscopic examination and the other half was fixed in 3% glutaraldehyde in 0.1 phosphate buffer solution for transmission electron microscopic examination [20]. Sections for light microscopic examination were stained with Hematoxylin and Eosin Stain and Masson's Trichrome Stain [21].

### 2.7 Statistical Study

All collected data of the weight and blood glucose was statistically analyzed. In this work, mean±standard deviation ( $\pm$  SD) and (P) values were calculated using Statistical Package for the Social Sciences (SPSS) version 20. (Non-significant P value > 0.05, \* Significant P value  $\leq$  0.05 & \*\* highly significant P value  $\leq$  0.001).

## 3. RESULTS

### 3.1 Body Weight Measurements

A highly significant decrease in the mean body weight of rats from both group III and IV was noticed after the 10<sup>th</sup> and after the 20<sup>th</sup> day of the

experiment compared to the control as shown in (Table 1).

### 3.2 Blood Glucose Measurements

#### 3.2.1 Comparison of blood glucose level between different groups

A highly significant increase in the blood glucose of rats from group III was noticed compared to the control at the end of the study. Diabetic rats treated with HBO from group IV showed a highly significant decrease in blood glucose as compared to group III at the end of the experiment. However, blood glucose in group IV was still highly significantly increased compared to control group (Table 2).

#### 3.2.2 Comparison between the blood glucose level in each group at different periods of the experiment

A highly significant increase was found in blood glucose level in group III after 10 days compared to the onset and a significant increase after 20 days compared to after 10 days indicating progressive increase. Group IV revealed highly significant increase after 10 days compared to

the onset but highly significant decrease after 20 days compared to after 10 days was manifest (Fig. 2).

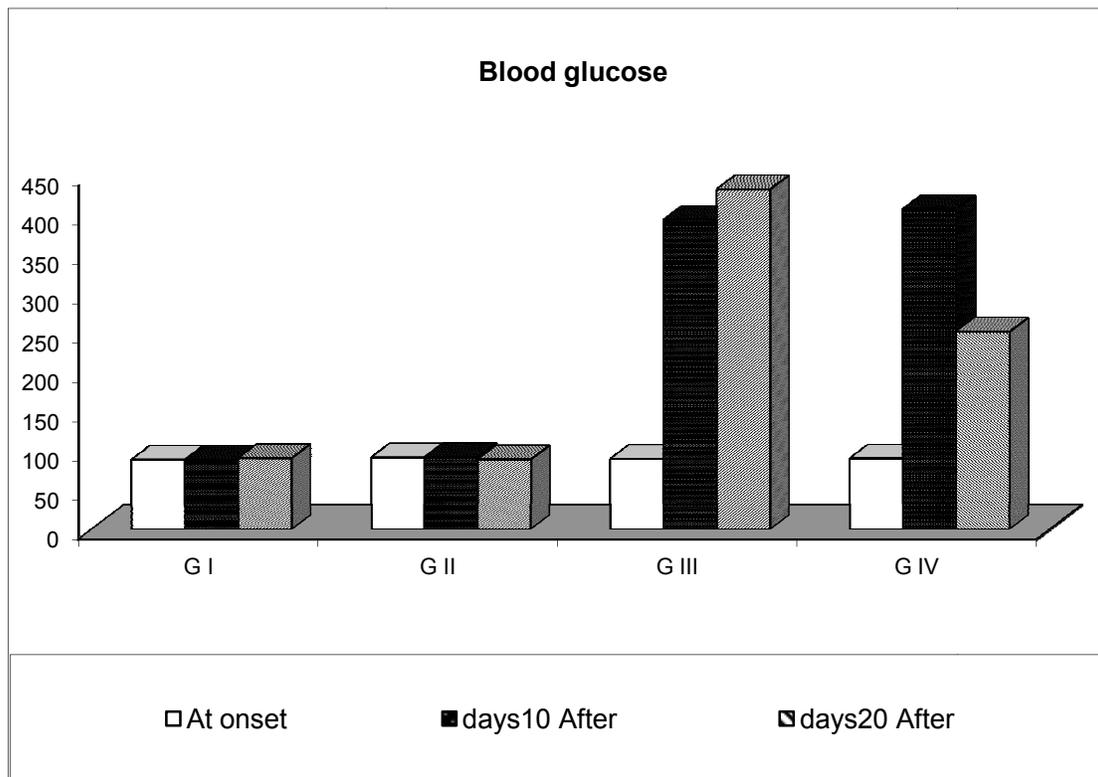
### 3.3 Histological Results

#### 3.3.1 Light microscopic examination of the liver (Figs. 3 and 4)

Haematoxylin and eosin stained sections of the liver of both control group and group II revealed the normal architecture of the hepatic lobule. Hepatocytes were arranged into cords radiating from a central vein. The hepatocytes appeared polyhedral with acidophilic cytoplasm and rounded vesicular nuclei. Blood sinusoids separating the cords of hepatocytes were lined with flat endothelial cells. Sections of group III revealed loss of the normal architecture of the hepatic lobule. The hepatocytes appeared vacuolated and irregularly arranged. Cellular infiltration was noticed around the dilated central vein and within the hepatic lobule. Distended hepatocytes with highly vacuolated cytoplasm

and central dark nuclei were seen. Some cells showed ballooning of the cytoplasm with formation of a big vacuole and the nuclei became peripheral and flattened.

Sections of group IV showed apparent restoration of the normal architecture of the hepatic lobule. Most of the hepatocytes were arranged into cords radiating from the mild congested central vein and separated by blood sinusoids. However, some cells were disrupted and irregularly arranged. Partially restored hepatocytes with deeply stained acidophilic cytoplasm and rounded vesicular nuclei were seen. However, some cells had small shrunken nuclei and others showed vacuolated cytoplasm. Masson's trichrome stained sections of the liver from group (I) and (II) showed the normal connective tissue distribution around the portal triad and between the hepatic lobules. Massive connective tissue infiltration was noticed around the portal triad and between the hepatic lobules in group III. Sections obtained from group IV showed reduction of connective tissue infiltration.



**Fig. 2. Comparison between the blood glucose level (mg/dl) of the four groups at different periods of the experiment**

**Table 1. Body weight measurements (gm) in all groups**

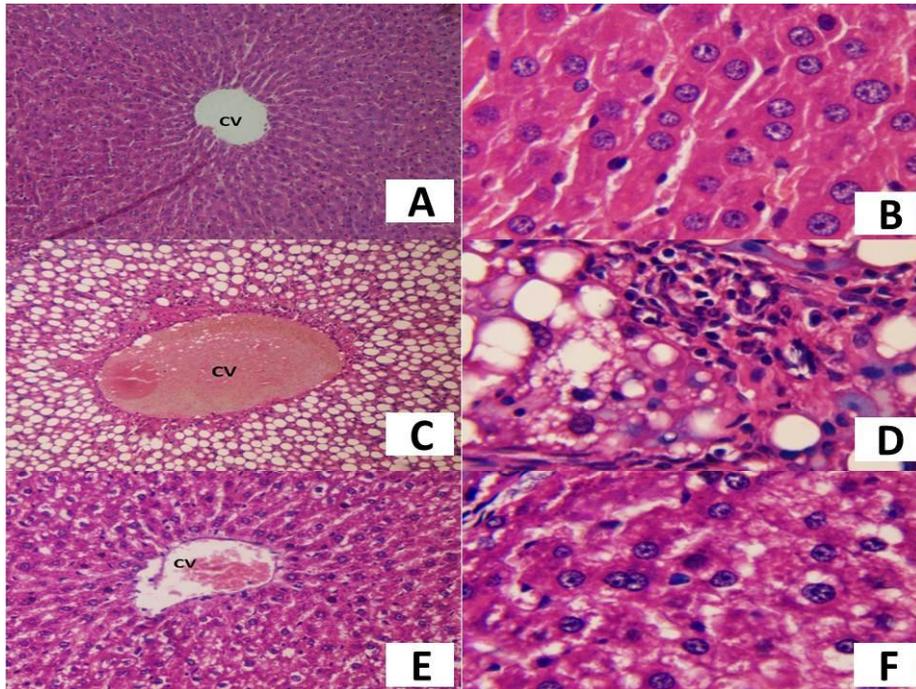
Weight		Range		Mean	±	S. D	F. test	P. value	Post hock test					
At onset	Non Diabetic	G I	180	–	220	195.63	±	12.66	0.092	0.964	P1	0.929	P4	0.722
		G II	180	–	220	196.25	±	14.58			P2	0.657	P5	0.929
	Diabetic	G III	180	–	220	198.75	±	14.58			P3	0.992	P6	0.657
		G IV	180	–	220	195.63	±	13.74						
After 10 days	Non Diabetic	G I	210	–	240	229.75	±	10.98	8.612	0.001*	P1	0.472	P4	0.002*
		G II	205	–	238	225.50	±	11.60			P2	0.001*	P5	0.007*
	Diabetic	G III	190	–	225	205.63	±	13.17			P3	0.001*	P6	0.640
		G IV	195	–	227	208.38	±	10.69						
After 20 days	Non Diabetic	G I	236	–	260	248.38	±	8.77	11.060	0.001*	P1	0.139	P4	0.002*
		G II	225	–	256	240.63	±	10.94			P2	0.001*	P5	0.009*
	Diabetic	G III	205	–	236	223.00	±	9.59			P3	0.001*	P6	0.529
		G IV	210	–	242	226.25	±	11.25						

P1: G I & G II, P2: G I & G III, P3: G I & G IV, P4: G II & G III, P5: G II & G IV, P6: G III & G IV, SD: standard deviation

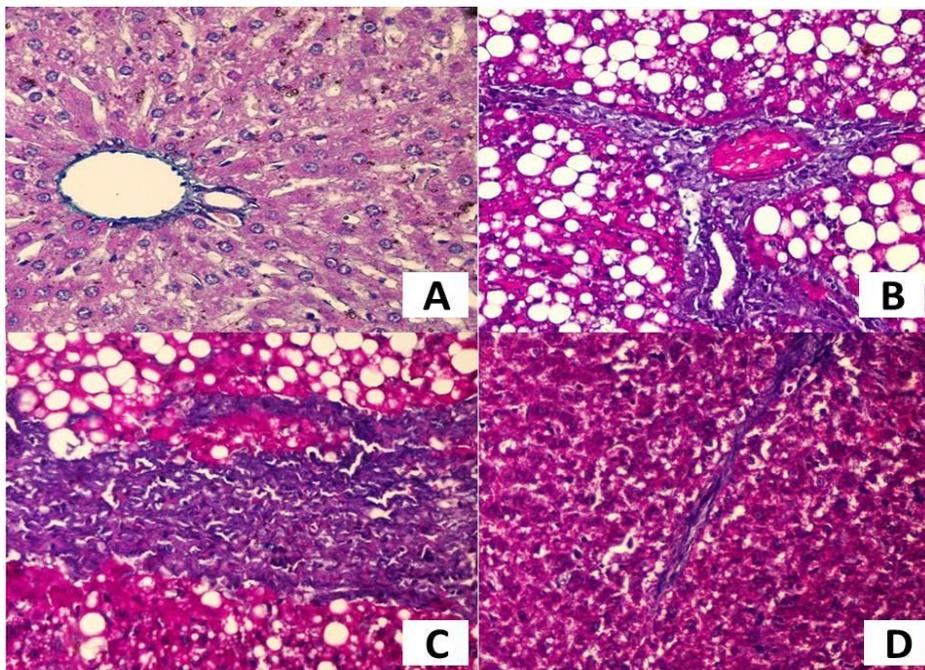
**Table 2. Blood glucose measurements (mg/dl) in all groups**

Blood glucose		Range		Mean	±	S. D	F. test	P. value	Post hock test					
At onset	Non Diabetic	G I	73	–	102	88.38	±	11.03	0.096	0.961	P1	0.604	P4	0.723
		G II	78	–	102	91.13	±	8.32			P2	0.869	P5	0.795
	Diabetic	G III	73	–	103	89.25	±	10.70			P3	0.795	P6	0.925
		G IV	73	–	107	89.75	±	11.57						
After 10 days	Non Diabetic	G I	73	–	102	87.88	±	10.83	190.319	0.001*	P1	0.850	P4	0.001*
		G II	76	–	105	91.38	±	9.58			P2	0.001*	P5	0.001*
	Diabetic	G III	310	–	460	393.25	±	55.71			P3	0.001*	P6	0.469
		G IV	315	–	466	406.75	±	45.74						
After 20 days	Non Diabetic	G I	74	–	105	90.13	±	11.87	243.963	0.001*	P1	0.960	P4	0.001*
		G II	75	–	104	89.38	±	10.76			P2	0.001*	P5	0.001*
	Diabetic	G III	349	–	485	432.00	±	46.76			P3	0.001*	P6	0.001*
		G IV	201	–	296	251.00	±	32.32						

P1: G I & G II, P2: G I & G III, P3: G I & G IV, P4: G II & G III, P5: G II & G IV, P6: G III & G IV, SD: standard deviation



**Fig. 3. Liver Hx. &E (A) & (B): Control; the hepatocytes are arranged into cords separated by blood sinusoids and radiating from the central vein (CV). (C) & (D): Group III; congested dilated CV, vacuolated cytoplasm of the hepatocytes and cellular infiltration. (E) & (F): Group IV; partial improvement, A, C & E x 200; B, D & E x 1000**



**Fig. 4. Liver Masson's trichrome X 400 (A): Control; normal connective tissue (CT). (B & C): group III; massive CT infiltration around portal triad and between the hepatic lobules. (D): group IV; minimal CT infiltration**

### 3.3.2 Light microscopic examination of the pancreas (Fig. 5)

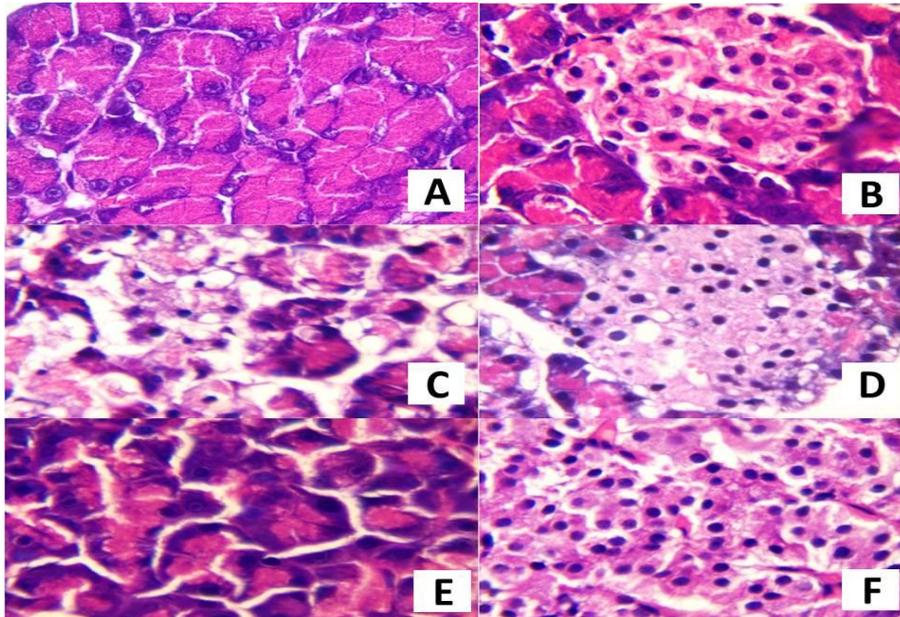
Haematoxylin and eosin stained sections of the pancreas in control group and group II showed the acini consisted of large pyramidal cells with apical densely stained acidophilic cytoplasm and basal basophilic nuclei surrounding a narrow lumen. The islet of Langerhans appeared as a rounded well demarcated mass of lightly stained acidophilic cells with rounded central nuclei. The islet cells arranged in branching and anastomosing cords separated by blood sinusoids. Group III showed multiple destructed acinar cells. Some acinar cells contained vacuolated cytoplasm surrounding shrunken nuclei. Loss of normal architecture of the islet of Langerhans was noticed. The cells were not arranged in anastomotic cords and blood sinusoids were hardly recognized. Some cells of the islet appeared distended with highly vacuolated cytoplasm. Other cells showed ballooned cytoplasm or possessed shrunken nuclei. In group IV some acini restored the normal pyramidal cells. Other acini showed cuboidal cells with wide lumen. Apparent restoration of the normal structure of the islet was seen. The islet was formed of branching and reanastomosing cords of cells that appeared lightly stained acidophilic with central nuclei.

However, some cells showed vacuolated cytoplasm with shrunken nuclei.

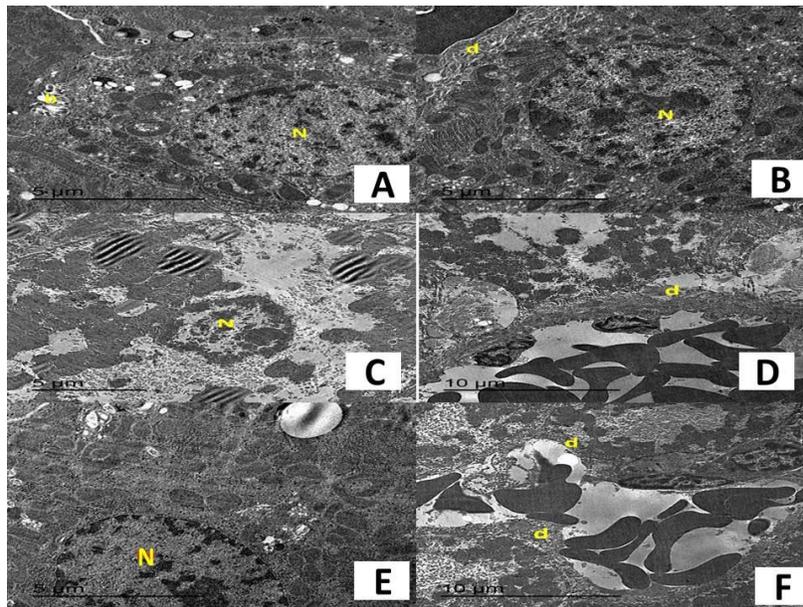
### 3.4 Ultrastructural Results

#### 3.4.1 Electron microscopic examination of the liver (Fig. 6)

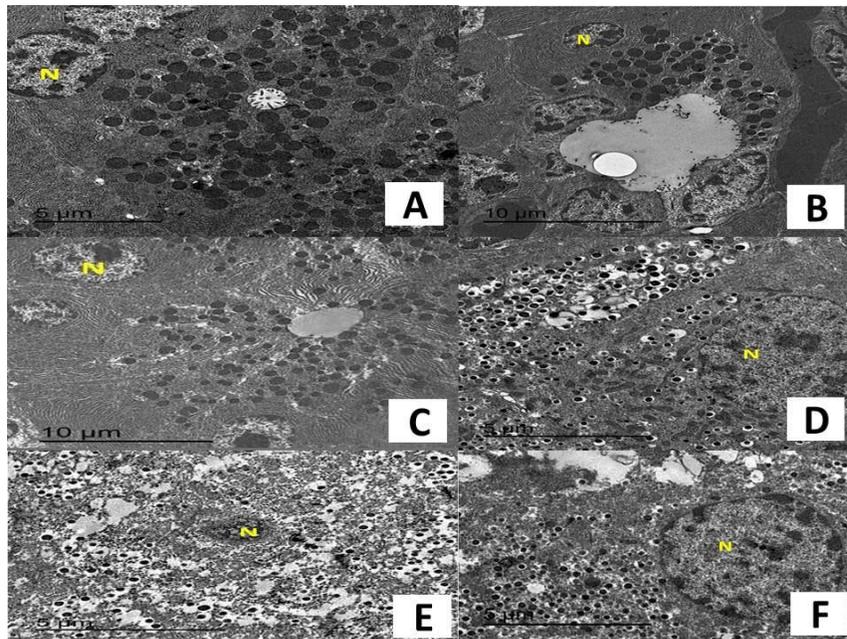
Ultrastructural examination of the liver in the control group and group II showed that the hepatocyte contained large euchromatic nucleus with prominent nucleolus, numerous cytoplasmic mitochondria and well developed rough endoplasmic reticulum. Bile canaliculi were seen between adjacent hepatocytes. Blood sinusoids containing RBCs with spaces of Disse between the sinusoid and the hepatocytes were seen. Hepatocytes in group III showed shrunken indented nuclei with condensed marginal chromatin and areas of rarified cytoplasm. Large lipid droplets, scattered mitochondria and depletion of glycogen granules were also seen. Deposition of excess collagen fibers in the space of Disse was noticed. Group IV showed the hepatocyte contained apparently normal euchromatic nucleus. The cytoplasm contained multiple mitochondria, some vacuoles and lipid droplets. The space of Disse showed less collagen fibers compared to group III.



**Fig. 5. Pancreas Hx. &E × 1000 (A&B): Control; Pancreatic acini (A) and islet of Langerhans (B). (C & D): Group III; destructed pancreatic acini and vacuolated cytoplasm of acinar cells (C), distended vacuolated islet cells (D). (E & F): Group IV; restoration of normal structure of acini (E) and islets (F)**



**Fig. 6. Liver. TEM (A&B): Control; normal hepatocyte, bile canaliculus (b) and space of Disse (d). (C) Group III; hepatocyte showing shrunken nucleus (N), rarified cytoplasm and lipid droplets. (D) Group III; deposition of excess collagen fibers in the space of Disse. (E& F) group IV; improved hepatocyte and space of Disse**



**Fig. 7. Pancreas TEM (A, B & C) Pancreatic acini, (D, E & F) Beta cells of islets of Langerhans. (A): Control; pancreatic acinus with pyramidal acinar cells, basal nuclei and apical zymogen granules. (B): Group III; cuboidal acinar cells with loss of apical zymogen granules and wide lumen. (C): group IV; improved pancreatic acini. (D): Control;  $\beta$  cell with normal nucleus and cytoplasmic granules surrounded by halos. (E): group III; pyknotic nucleus, vacuolated cytoplasm and apparent reduction of cytoplasmic granules. (F): group IV; normal nucleus, restored cytoplasmic granules and vacuolated cytoplasm**

### 3.4.2 Electron microscopic examination of the pancreas (Fig. 7)

The pancreatic acini of rats from group I and group II showed the acinus cell that was pyramidal in shape and contained a basal nucleus. Its cytoplasm showed abundant apical zymogen granules and well developed rough endoplasmic reticulum. The apical surface of these cells faced the lumen of the acinus and showed microvilli. Some acinar cells in Group III lost the normal pyramidal appearance and appeared cuboidal with central oval nuclei and absent zymogen granules. The lumen of the acinus appeared wide with irregular outline. In group IV the acinus cell regained its pyramidal shape and contained a basal euchromatic nucleus. The cytoplasm showed numerous apical zymogen granules and extensive rough endoplasmic reticulum.

Beta cells in the islet of Langerhans in the control group and group II showed that it contained euchromatic nucleus with prominent nucleolus and numerous cytoplasmic electron dense granules surrounded by wide electron lucent halo. Multiple mitochondria and rough endoplasmic reticulum were also noticed. Group III showed multiple vacuoles in the cytoplasm of beta cells and increase in the halo spaces around the electron dense granules. Shrunken condensed nucleus and swollen mitochondria with disrupted cristae and vesicles inside were noticed. Apparent reduction in the number of electron dense granules was seen. In group IV beta cells showed apparently normal euchromatic nuclei. The cytoplasm showed apparent increase in the number of secretory granules surrounded by normal lucent halos. Some vacuoles were seen.

## 4. DISCUSSION

The last few years showed the wide use of HBOT in treatment of many conditions. Its role in improving hyperglycemia in type 2 diabetes has been proved in previous researches [13]. In this study, a diabetic rat model has been established by intraperitoneal injection of dexamethasone for a period of ten days. HBOT was given to diabetic rats for successive 5 sessions in the period from the 11<sup>th</sup> day till the 20<sup>th</sup> day.

In this work, no significant differences in blood glucose were noticed between control and HBO treated non diabetic groups in all experimental periods. Similar findings were reported in

previous work [22]. A highly significant increase in blood glucose was noticed after 10 days injection of dexamethasone in group III and IV compared to the control rats. Hyperglycemia after dexamethasone treatment was a common outcome of previous studies [23,24]. At the end of the experiment, group IV rats revealed a highly significant decrease in blood glucose as compared to group III denoting regression of blood glucose with HBOT. However, the blood glucose level was still significantly higher in diabetic HBO treated rats compared to the control. This means that HBO therapy decreases the level of blood glucose. However, normal blood glucose levels haven't been reached. Previous researches explained that, HBOT decreases hyperglycemia through regeneration of pancreatic  $\beta$ -cells and increasing insulin sensitivity. They also added that, it inhibits diabetic complications as diabetic nephropathy, endothelial cell damage and poor wound healing [13,25]. However, the findings of other authors wherein contrast with our results. They suggested that HBOT should be used cautiously in diabetic patients as it enhanced the cytotoxic effect on the beta cells of the pancreas. They also added that, increased blood glucose and decreased beta cells were demonstrated in diabetic HBO treated rats [26].

In this work, no significant difference was observed in the body weight in group (II) at the end of the experiment as compared to the control group. The same results were found in previous literature [1]. In contrast with our results, some reported decrease in weight after HBOT compared to the control group owing to increasing oxygen supply and systemic metabolism [8]. A highly significant decrease in the body weight of untreated diabetic rats from group III was seen after the 10<sup>th</sup> and the 20<sup>th</sup> day of the experiment compared to the control group. Polyphagia with highly significant decrease in the body weight in untreated diabetic rats compared to the control were noticed. This may be explained by catabolic processes, hypoinsulinemia that led to increased appetite owing to sense of energy deficiency caused by disturbed glucose transport [27]. At the end of this study, the body weight of the HBO treated diabetic rats (group IV) showed highly significant decrease compared with the rats of group I. similar findings were demonstrated previously [22]. It was suggested that weight loss enhanced insulin sensitivity [28] and this is one of the mechanisms by which HBOT is effective in treatment of type 2 diabetes [25].

The histological and ultrastructural results of sections of the liver from group II showed the normal liver architecture as that of the control group. Some researchers reported that HBOT didn't alter the normal histology of the liver [29, 30]. However, others demonstrated clustered hepatocytes around the central vein, tortuous sinusoids and vacuoles around the nuclei in the liver after HBOT [31].

Sections of the liver from group III revealed obvious changes indicating hepatic steatosis and liver fibrosis. The hepatic lobules appeared distorted with disorganized hepatocytes that were distended with highly vacuolated or ballooned cytoplasm. Extensive connective tissue infiltration was noticed between the hepatic lobules, around the central vein and around the portal triad. These findings were supported by the work of other researchers [32] who suggested that changes in the liver parenchyma started with microvesicular steatosis then macrovesicular steatosis followed by cirrhosis and hepatocellular carcinomas with end stage liver failure. Some authors showed that diabetic liver increased lipid hydroperoxide oxidant radicals production that decreased the activity of antioxidant enzymes. Thus, diabetes caused oxidative damage in the liver, non-alcoholic fatty liver (NAFL) and hepatocellular carcinoma. NAFL resulted from imbalance in the uptake, synthesis and oxidation of free fatty acids as the liver is the main organ in lipogenesis and cholesterol metabolism [33,34].

The ultrastructural examination of the liver from group III diabetic non treated rats showed marked damage of the hepatocytes. They contained shrunken indented nuclei and their cytoplasm showed many large lipid droplets, scattered disrupted mitochondria, depletion of glycogen granules and slightly dilated rough endoplasmic reticulum. The space of Disse showed excess collagen fibers. This was explained by some authors as [32,35] who explained that diabetes caused degeneration of the hepatocytes which contained pyknotic nuclei with abnormalities of the nuclear membrane. They added that, there was marked reduction in the number of cytoplasmic organelles, loss of mitochondrial cristae and increased lipid accumulation.

Group IV rats revealed partial restoration of the normal architecture of the liver and structure of the hepatocytes. Also, it showed decreased connective tissue infiltration between the hepatic lobules and around the portal triad compared

with group III. These findings were in agreement with other researchers [36,37] who reported that HBO treatment improved liver functions and stimulated hepatocyte regeneration in cases of liver cirrhosis. Also, our results were supported by the work of [38,39] who demonstrated the protective effects of HBOT on the liver and some of them attributed these effects to the anti-inflammatory and antioxidant action of HBOT. In contrast with our results, some authors reported that HBOT increases oxidative stress that leads to more hepatocellular damage [8]. They added, HBOT reduces fat accumulation in the liver, causes deterioration of cellular damage and increase in serum triglycerides and low density lipoprotein (LDL).

Samples of the pancreas from group II showed the same normal pancreatic structure as found in the control group. These results were in agreement with [26,40] who demonstrated the normal histological structure of the pancreas in both control and HBOT rats. Some authors reported that chronic treatment with HBO in prediabetic mice or after islet cells transplantation prevented development of diabetes as it preserves the normal histological structure and function of the pancreas [41].

Histological examination of the pancreas from group III revealed disorganized architecture of the pancreatic tissue with intralobular haemorrhage. Disrupted pancreatic acini and intralobular cellular infiltration were seen. The cells of the pancreatic acini lost their pyramidal appearance and appeared cuboidal. These findings were in agreement with [42,43] who reported that diabetes mellitus impaired both histology and function of the pancreatic acini. They added that, signs of atrophy of the pancreatic acini with loss of the normal structure of acinar cells were noticed. This was attributed to insufficient insulin reaching the pancreatic acini which in turn led to decrease in the pancreatic size and finally its atrophy.

The cells of the islets of Langerhans in group III showed loss of the normal arrangement. Their cytoplasm contained many vacuoles and some cells showed ballooning. Previous researches reported that diabetic rats revealed pathological changes in both pancreatic acini and islets of Langerhans [44,45]. They added that the acinar cells were damaged and contained vacuolated cytoplasm with degeneration and downregulation of beta cells.

Some cells of the pancreatic acini from group III appeared cuboidal with absent zymogen granules by electron microscopic examination. The cytoplasm of beta cells in the islets of Langerhans showed multiple vacuoles and swollen and degenerated mitochondria. Reduction of the number of the cytoplasmic electron dense granules was also noticed. Multiple shrunken nuclei were observed. This was in accordance with previous work [46] which revealed that diabetes resulted in marked pathological changes in the pancreas in the form of vacuolated cytoplasm, decreased secretory granules, damaged mitochondria and dilated rough endoplasmic reticulum in the acinar cells and decrease of secretory granules and pyknotic nuclei of the beta cells. Many authors demonstrated the therapeutic effect of HBO in cases of diabetes mellitus and some attributed this to its effect on the pancreas, stimulating insulin secretion and improving insulin resistance in diabetic patients [47,48]. The present study showed apparently improved histological and ultrastructural architecture of the pancreas of the diabetic HBO treated rats (group IV) compared to the diabetic non treated rats (group III). Apparently normal pancreatic acini and islets of Langerhans were demonstrated in most of the sections.

Ultrastructural study for pancreas obtained from group IV rats in this study showed that the acinar cells regained their pyramidal appearance and contained basal euchromatic nuclei. The cytoplasm showed numerous apical zymogen granules. Some beta cells showed apparently normal euchromatic nuclei but other cells contained shrunken nuclei with areas of chromatin clumping. The number of secretory granules in  $\beta$  cells was apparently increased compared to group III. Previous studies reported that HBO treatment was associated with improvement of the pancreatic structure and regeneration of pancreatic  $\beta$ -cells in cases of diabetes mellitus [13]. Others used HBOT in treatment of acute pancreatitis and they found that it improved oxygen supply of tissues, maintained cellular integrity and helped regeneration of pancreatic tissues [49].

## 5. CONCLUSIONS

The present study found that HBOT is partially effective in reducing blood glucose level and improving the hazardous effect of diabetes on the histological and ultrastructural architecture of the liver and pancreas of male albino rats. More

studies are recommended to evaluate the use of HBOT as an adjuvant therapy with other antidiabetic treatments.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All the steps of the experiment were carried out according to the standard guidelines for animal care and rights that was approved by the ethical committee on animal's experiments of Tanta University.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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