Histological and Immuno-Histochemical Study on the Possible Effect of Vitamin K2 on the Hepatic Oval Cells in Diabetic and Non-Diabetic Albino Rats

Original Article

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ABSTRACT

Background: Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from hypoinsulinemia, defective insulin action, or both. ß-cells regeneration from stem cells or pancreatic progenitor cells is an attractive method for diabetic treatment. Hepatic oval cells (HOCs), intra-hepatic cells of bi-potent progenitor capabilities, can proliferate and differentiate into both hepatocytes and bile duct epithelial cells. They had the potentiality to transdifferentiate into pancreatic endocrine cells, as both liver and pancreas have the same origin (upper primitive foregut endoderm). Vitamin K2 can promote recovery in patients with liver cirrhosis and prevent insulin resistance reducing the risk of type 2 diabetes.

Aim of the work: Evaluating the histological and immunohistochemical changes in hepatocytes and oval cells in diabetic and non-diabetic rats' liver under treatment with vitamin K2.

Materials and methods: 88 adult male albino rats were divided into 3 groups: Group I (control group), Group II (diabetic group); where diabetes was induced using single intraperitoneal (IP) dose of streptozotocin (STZ) following 2 weeks of high fat diet and Group III (treatment group) where diabetic rats were given daily oral vitamin K2 for 20 days (5 days on and 5 days off). Random blood sugar was measured for each group at day 0, 16 and then every 5 days till the end of the experiment (36 days). All groups' sections were subjected to H&E and immunohistochemical stains for CD34, CK19 and insulin. Optical density for CD34 positive immunoreaction and mean area percent for CK19 and insulin immunoreaction were measured and data were statistically analyzed.

Results and Conclusion: HOCs gave a positive reaction of changing into insulin producing cells in diabetic rats thus they may be modified to change into extra pancreatic source of insulin. Additionally, administration of vitamin K2 helped increase the proliferative ability of the HOCs in case of diabetes.

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Key Words: CK19, diabetes, hepatic oval cells, streptozotocin, vitamin K2.

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INTRODUCTION

Diabetes mellitus comprises a group of heterogeneous disorders, which have hyperglycemia in common. It can be classified into 4 types (type 1, type 2, gestational diabetes and diabetes of specific causes). Type 2 diabetes is characterized by progressive loss of insulin secretion on the background of insulin resistance[1].

Diabetes, especially type 2, is considered as the most common cause of liver diseases and transplantation in U.S.A.[2]. Such diseases include non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, and acute liver failure. In addition, there is an unexplained association of diabetes with hepatitis C. The prevalence of diabetes in cirrhosis is 12.3–57%[3].

The replacement or restoration of inadequate beta cells would be considered as therapeutic options for diabetes. Nowadays, multiple studies using various strategies have shown that differentiation of stem and progenitor cells to insulin-producing cells will be the best future categories for treatment of diabetes[4].

Hepatic oval cells play a critical role in liver regeneration after injury. Their activation was detected under chronic liver injury caused by inflammation, chronic alcoholism-induced cirrhosis, and in patients with chronic hepatic necrosis, as well as in different animal models of liver injury[5].

Recently, spontaneous expression of extra-pancreatic proinsulin-producing beta-cells in STZ-induced diabetic animals has been reported in multiple organs of mice and rats[6]. These cells can be detected in liver, adipose tissue, spleen, bone marrow, and thymus[7].

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Additionally, administration of vitamin K2 has been proved to have a hepato-protective effect through the maintenance of hepatic oval cells in the liver of diabetic subjects[8].

MATERIALS AND METHODS

Animals:

Eighty eight adult male albino rats, weighed 240 - 260g, were used in this study. They were housed in the Animal House, Faculty of Medicine, Cairo University. The rats were treated according to the guidelines approved by the Animal Use Committee of Cairo University. Rats were provided with ordinary rat chow, bred and housed in wire mesh cages at temperature (24±1°C), with normal light/dark cycle. All animals were kept under the same environmental conditions and had free access to water and food before use in experimental protocols.

Chemicals:

- Streptozotocin (STZ) an antibiotic: it was purchased from Sigma Company (St. Louis, Mo, USA) in the form of powder. It was dissolved in sodium citrate buffer[9].

-Vitamin K2: it was purchased from Anhui Wanhe Pharmaceutical Co., LTD in powder form. It was dissolved in sun flower oil[8].

- Sunflower oil: it was purchased from Imtinan, Cairo ARE.

- Chemicals for immunostaining:

Primary antibodies:

CD34 Ab-1 (Clone QBEnd/10): It is a mouse monoclonal antibody (Lab Vision Corporation laboratories, CA 94539, USA, catalogue number MS-363-R7).

Cytokeratin 19 (CK19) Polyclonal Antibody: It is a rabbit polyclonal antibody (proteintech Corporation laboratories, USA, catalogue number 10712-1-AP).

Insulin Ab-6 (INS04+INS05): It is a mouse monoclonal antibody (Lab Vision Corporation laboratories, CA 94539, USA, catalogue number MS-1379-P).

Citrate buffer (cat no AP 9003), Ultravision detection system (biotin-streptavidin detection system, cat no TP - 015- HD) and Mayer's hematoxylin (cat no TA- 060- MH). All were purchased from Labvision Thermoscientific, USA.

Experimental design:

Random blood sugar was done for all animals, after making sure that all rats were not diabetics with RBS < 200 mg/dL. They were divided into 3 groups:

Control group (group I, 48 rats):

They were subdivided equally into four subgroups, 12 rats each:

Subgroup Ia: rats had free access to water and food without receiving any treatment for the whole experimental duration (36 days).

Subgroup Ib: animals had free access to water and food for 2 weeks then each rat was given a single IP injection of 1ml sodium citrate buffer on day 14.

Subgroup Ic: the rats were treated as in subgroup Ib. Two days later (day 16), they were given 1ml sun flower oil daily orally via a gastric tube for 20 days (5 days on & 5 days off).

Subgroup Id: the rats were treated as in subgroup Ib. Two days later (day 16), they were given daily orally vitamin K2 (15mg/kg) dissolved in 1ml sun flower oil/ rat, via a gastric tube for 20 days (5 days on & 5 days off).

Diabetic group (group II, 20 rats):

Diabetes was induced in the rats of this group. On day 16, diabetes was confirmed, then the rats were given nothing till the end of the experiment.

Treatment group (group III, 20 rats):

On day 16, after induction and confirmation of diabetes as in group II, the rats of this group received, via a gastric tube, daily oral vitamin K2 (15 mg/kg) dissolved in 1 ml sunflower oil for each rat, for 20 days (5 days on & 5 days off)[8].

Three rats from each control subgroup together with 5 rats from groups II and III were sacrificed using decapitation method (Guidelines for Proper Conduct of Animal Experiments, 2006) at these time points; day 21, 26, 31 & 36 of the experiment.

Experimental procedure:

Measuring the body weight:

The body weight of all animals was measured using the scale, at the start of the experiment, on day 16 and at each time point just before sacrifice at the Animal House, Faculty of Medicine, Cairo University.

Induction of diabetes (14 days):

Diabetes was induced through a single intraperitoneal injection of STZ (30 mg/kg) dissolved in 1ml sodium citrate buffer for each rat after being fed with a high fat diet for 2 weeks (42% energy; fat sources: sheep fat tail)[9,10]. Two days after STZ administration (day 16), random blood sugar was measured to confirm diabetes, the animals were considered diabetic if their blood glucose level was higher than 200 mg/dL[11].

Biochemical investigation:

Random blood sugar was measured at each time point just before rats' sacrifice at Biochemistry department, Faculty of Medicine, Cairo University. The blood samples were obtained from the retro orbital vein in collecting heparinized capillary tubes. The samples were centrifuged at 1000g and plasma was obtained and frozen

in polypropylene tubes at −20°C until assayed[11].

Light microscopic studies:

The livers of the rats of each control subgroup and experimental group were fixed in 10% formol saline and kept for 24 hours then processed to obtain Paraffin blocks. Paraffin sections 6 micrometres thick were cut and stained by:

- Hematoxylin and Eosin stains (H&E)[12].
- Immunohistochemical staining for:
    a. CD34, a marker for endothelium of the portal tract and HOCs.
    b. CK19, a marker for keratin present in the epithelial cells of the bile ducts and HOCs.
    c. Insulin, a marker for insulin producing cells.

Immunostaining required pretreatment[13], this was done by boiling for 10 minutes in 10Mm, pH 6 citrate buffer for antigen retrieval and leaving the sections to cool in room temperature for 20 minutes. Then, the sections were incubated for one hour with the primary antibodies. Immunostaining was completed by the use of Ultravision detection system and counterstaining was done using Mayer's hematoxylin.

Morphometric study:

It included measuring of:

- Optical density of CD34 immuno-positive cells in anti-CD34 immunostained sections at a magnification of ×400.
- Mean area percent of CK19 positive immuno-reactivity in anti-CK19 immunostained sections at a magnification of ×400.
- Mean area percent of insulin positive immune-reaction in anti-insulin immunostained sections at a magnification of ×400.

All measurements were done in 10 non overlapping fields from different sections of each control subgroup and each experimental group. Image analysis was done using "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, England) at Histology Department, Faculty of Medicine, Cairo University.

Statistical analysis:

The body weight, morphometric and biochemical measurements were expressed as mean ± standard deviation (SD) and were analyzed statistically using the software "Statistics for windows SPSS" version 19 (Chicago, USA). This was done using one-way analysis of variance ANOVA followed by "Tuckey" post hoc test. Results were considered statistically significant $P < 0.05$[14].

RESULTS

General observation:

No deaths were observed throughout the whole duration of the experiment.

All control subgroups revealed similar biochemical and histological results. Thus, they were collectively referred to as control group (group I).

Statistical analysis of the mean body weight (Table 1):

Mean body weight revealed significant gradual increase which was within the normal range for age in group I. In groups II & III, there was significant decrease versus control group starting from day 16 of the experiment. Additionally, there was significant increase in group III compared to group II in days 26, 31 & 36. Moreover, significant gradual decrease in body weight in group II and III versus group I, starting from day 21 of the experiment was noticed. The rate of body weight decrease was slower in group III when compared to group II.

Statistical analysis of the biochemical results (Table 2):

Mean random blood sugar level revealed relatively stable blood glucose level within the normal range for age in the group I, while there was sudden increase in blood glucose level on day 16 of the experiment in groups II and III. Additionally, there was significant increase in group II (70% of rats) & III (40% of rats) versus control group and significant decrease in group III compared to group II at each time point. Moreover, significant gradual increase in the mean value of random blood sugar was noticed in group II starting from day 16 to day 36, while in group III there was significant gradual increase from day 16 to day 26 then significant gradual decrease from day 26 to day 36.

Histological results:

Light microscopic results:

H&E stained sections:

Control group (group I):

Examination of the liver sections showed normal histological appearance of the classic hepatic lobule with a central vein and few mononuclear cell infiltration (MNCI) at its center. Cords of hepatocytes were noticed radiating from the central vein and separated by blood sinusoids that were lined by endothelial cells. The hepatocytes appeared to be polyhedral acidophilic cells with a central rounded vesicular nuclei while few of them were binucleated. The portal tract at the corners of the hepatic lobule consisted of a branch of the hepatic artery, a branch of the portal vein and a bile duct (Figs. 1a & 1b).

Diabetic group (group II):

Liver sections of this group from day 21 up to day 36

of the experiment revealed MNCI at the periphery of the classic hepatic lobule. Some of the hepatocytes at the center and the periphery of the classic hepatic lobule appeared with rarefaction of the cytoplasm, others showed deeply acidophilic cytoplasm and pyknotic nuclei. Additionally, congestion of the blood sinusoids and portal vessels was noticed (Figs. 1c, 1d, 1e & 1f).

Treatment group (group III):

Throughout the whole experimental duration, examination of the liver sections from group III showed MNCI in-between the hepatocytes' rows and at the center and the periphery of the classic hepatic lobule. As for the hepatocytes, there was rarefied (less than in group II) and/or deeply acidophilic cytoplasm and pyknotic nuclei. Congestion of the sinusoids and the branches of the portal veins was noticed in some sections (Figs. 1g, 1h, 1i & 1j).

Immunohistochemical stained sections:

Anti- CK19:

Examination of the liver sections demonstrated negative immunoreaction in group I (Fig. 2a).

In group II, positive cytoplasmic reaction in the cellular infiltration on day 21, 26 and 31 of the experiment was detected (Figs. 2b, 2c & 2d) and negative immunoreaction on day 36.

In group III, liver sections demonstrated positive cytoplasmic reaction in some cells at the periphery of the hepatic lobule on day 21, 26, 31 & 36 of the experiment (Figs. 2e, 2f, 2g & 2h).

Knowing that CK-19 is one of the markers of the hepatic oval cells so those immune-positive cells are hepatic oval cells.

Anti-CD34:

Examination of immune-stained liver sections demonstrated negative immunoreaction in group I (Fig. 3a).

In group II, positive cytoplasmic reaction in some cells at the periphery of the hepatic lobule around the portal tract on day 26 and 31 of the experiment was detected (Figs. 3b & 3c) and negative immunoreaction on day 21 & 36.

In group III, liver sections demonstrated positive cytoplasmic reaction in some cells at the periphery of the hepatic lobule on day 26, 31 & 36 of the experiment (Figs. 3d, 3e & 3f) and negative immunoreaction on day 21.

Knowing that CD34 is a marker for hepatic oval cells so the immune positive cells are hepatic oval cells.

Anti-insulin:

Examination of immune-stained liver sections demonstrated negative immunoreaction in group I (Fig. 4a).

In group II, positive cytoplasmic reaction at the periphery of the hepatic lobule around the portal tract on day 31 of the experiment was detected (Fig. 4b) and negative immunoreaction on day 21, 26 & 36.

In group III, liver sections demonstrated positive cytoplasmic reaction in some cells at the periphery of the hepatic lobule on day 21, 26 & 31 of the experiment (Figs. 4c, 4d & 4e) and negative immunoreaction on day 36.

Morphometric results (Table 3):

Mean area percent of CK19 immuno-positive cells:

It showed significant increase in group III versus group II.

Mean optical density of CD34 immune-positive cells:

Group III demonstrated significant increase when compared to group II.

Mean area percent of insulin immuno-positive cells:

There was significant increase in treatment group compared to diabetic group.

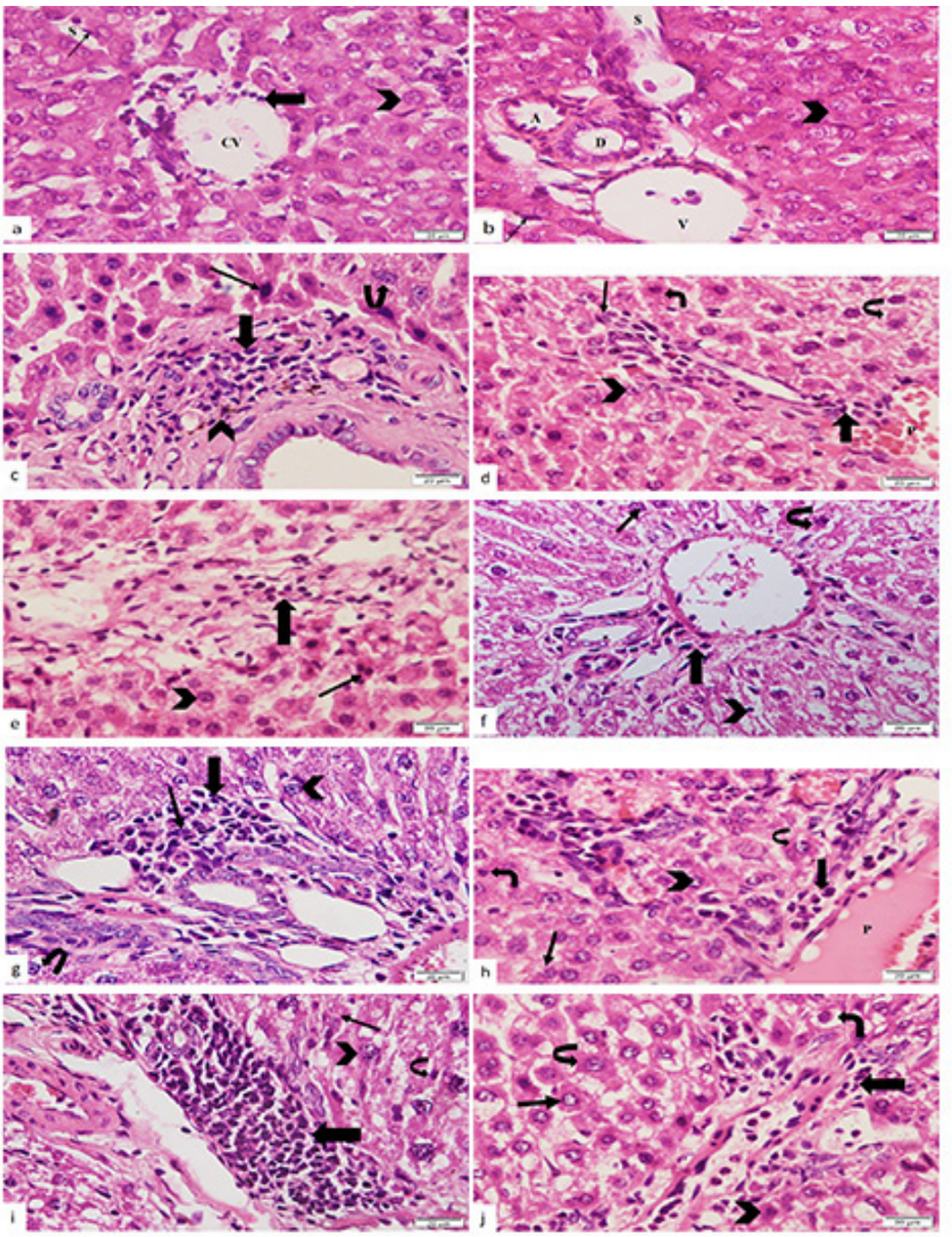

Fig. 1: Photomicrographs of liver sections showing: a & b: polyhedral hepatocytes with acidophilic cytoplasm and vesicular nuclei (arrow head). They are arranged as cords separated by blood sinusoids (S) lined by endothelium (thin arrow). These cords appear radiating from the central vein (CV). There is minimal MNCI around the central vein (thick arrow). the portal tract containing a hepatic artery (A), portal vein (V) and a bile duct (D) can be also seen. c: group II (day 21), shows shrunken hepatocyte with condensed, pyknotic nuclei & deeply acidophilic cytoplasm (thin arrow). There is also MNCI around thick walled bile duct (thick arrow). Some hepatocytes appear nearly normal (curved arrow). Haemosiderin granules can be seen (arrow head). d: group II (day 26), there is MNCI in the portal area (thick arrow), some of the cells of the infiltrate are basophilic with vesicular nuclei (thin arrow). Some hepatocytes appear normal with vesicular nuclei and apparent nucleoli (arrow head), some possess deep acidophilic cytoplasm (right-angled arrow) and some have rarified cytoplasm (curved arrow). There is congestion of portal vessels (P). e: group II (day 31), MNCI in the portal area (thick arrow). Hepatocytes appear normal with vesicular nuclei and prominent nucleoli (arrow head) &some cells show deep acidophilic cytoplasm and pyknotic nuclei (thin arrow). f: group II (day 36), MNCI in the portal areas (thick arrow). Hepatocytes show rarified granular cytoplasm (curved arrow), some have vesicular nuclei (thin arrow) & others hepatocytes show pyknotic nuclei (arrow head). g: group III (day 21), there is MNCI in the portal area (thick arrow), with basophilic cells and vesicular nuclei among the infiltrate (thin arrow). Hepatocytes possess vesicular nuclei (arrow head) while some show rarefied granular cytoplasm (curved arrow). h: group III (day 26), MNCI in the portal area (thick arrow) with congested branches of the portal vein (P) are detected. Hepatocytes possess vesicular nuclei (arrow head), some show vacuolations (thin arrow), some have dark acidophilic cytoplasm (curved arrow) &others show apoptotic changes (right-angled arrow). i: group III (day 31), extensive MNCI in the portal area is detected (thick arrow). Hepatocytes possess vesicular nuclei (arrow head), some hepatocytes show vacuolations (thin arrow) &others show apoptotic changes (curved arrow). j: group III (day 36), MNCI in the portal area & between hepatocytes (thick arrow) is noticed. Hepatocytes possess vesicular nuclei (thin arrow), many cells have deeply acidophilic cytoplasm (curved arrow), some show vacuolation (right-angled arrow) &others show apoptotic changes (arrow head). [H&E, x400]

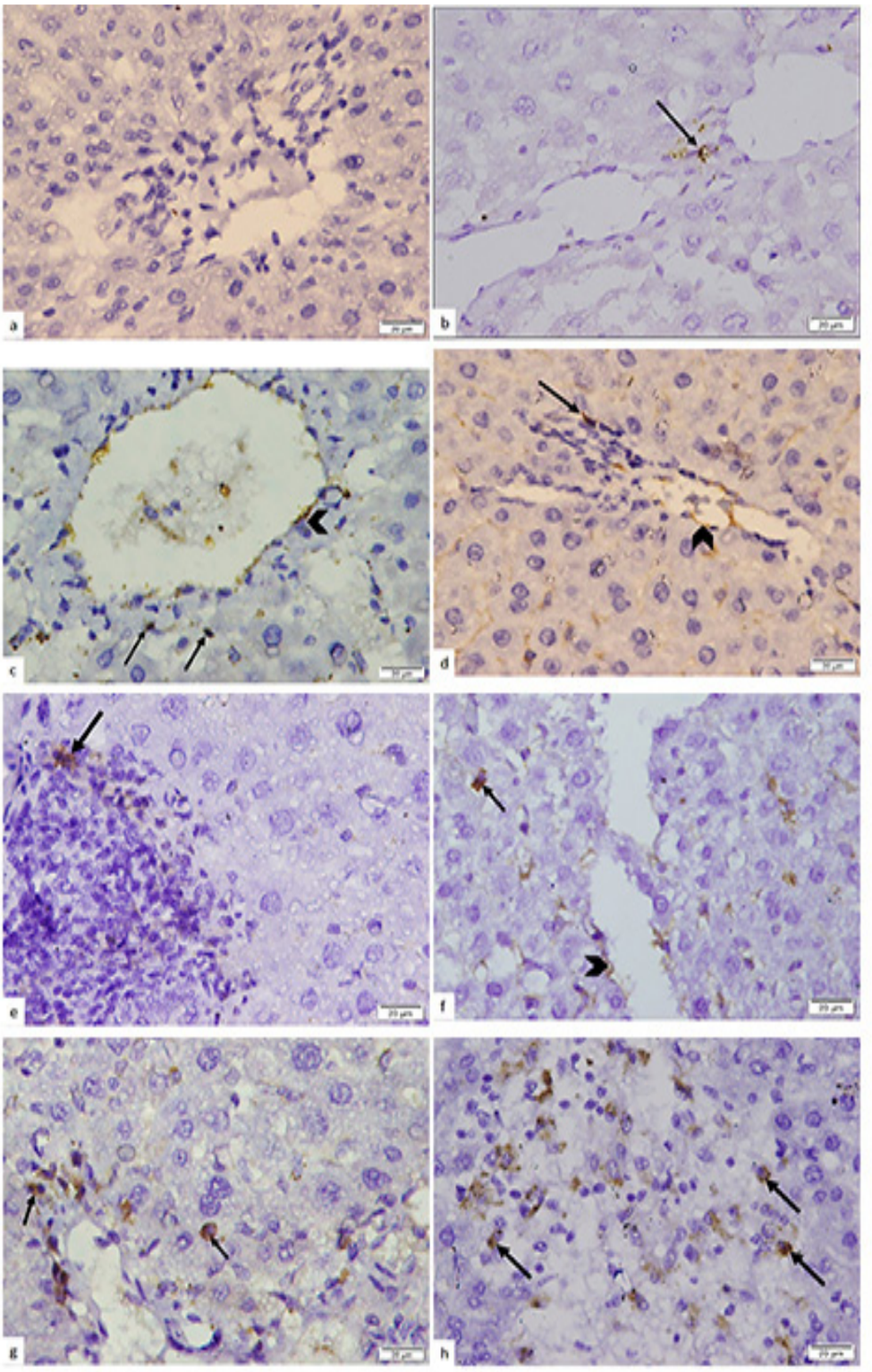

Fig. 2: Photomicrographs of liver sections showing: a: negative immunoreaction for CK19 in group I. b, c &d: group II (day 21, 26 & 31), positive cytoplasmic immune-reactivity for CK19 in the oval cells (arrow) at the periphery of the hepatic lobule around the portal tract can be detected. Group II (day 36), shows negative immunoreaction for CK 19 (not included in the figure). e, f, g & h: group III (day 21, 26, 31 & 36), shows positive cytoplasmic immunoreactivity for CK19 in the oval cells (arrow) at the periphery of the hepatic lobule around the portal tract. [CK19 immune-staining, x400]

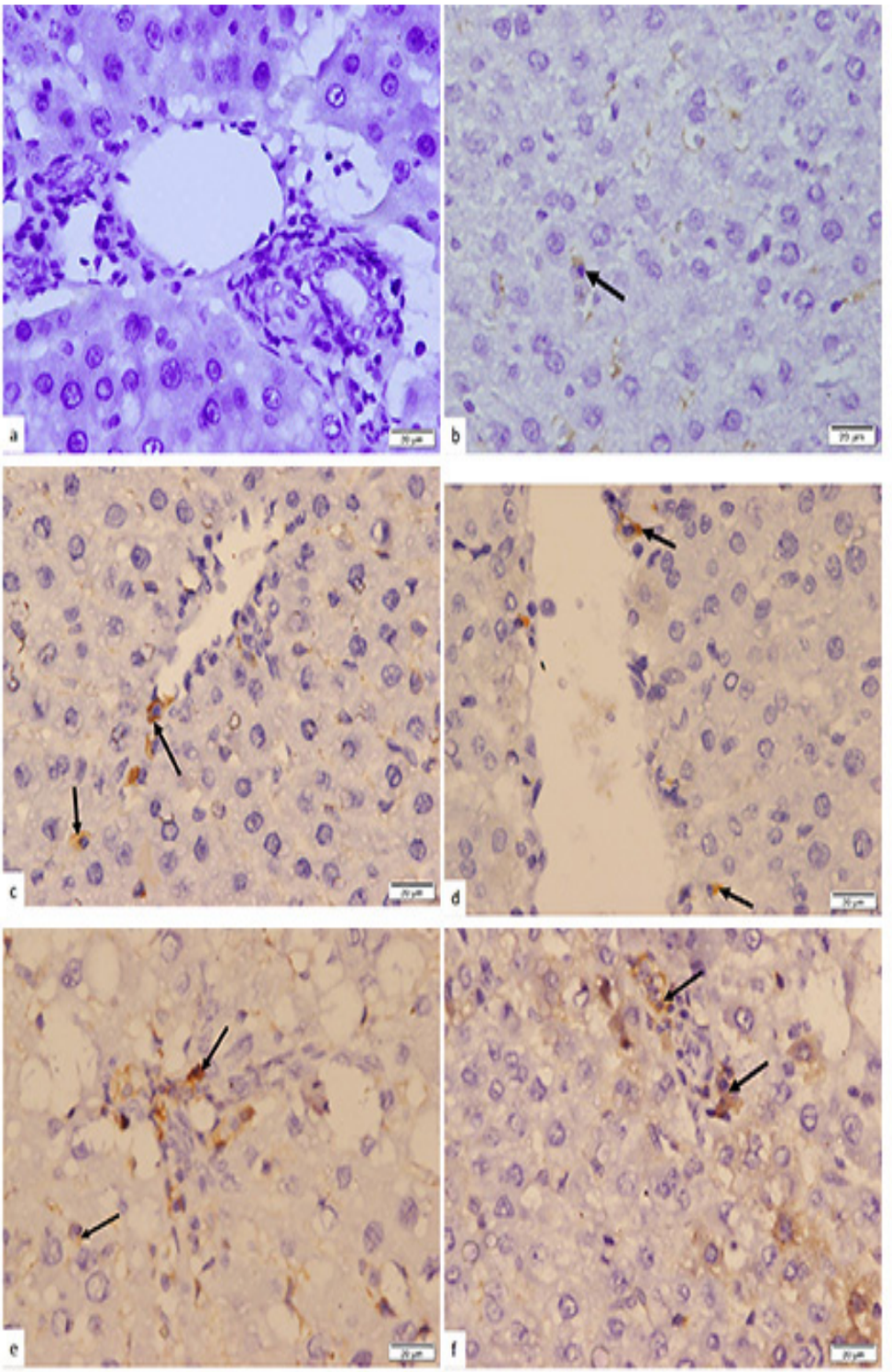

Fig. 3: Photomicrographs of liver sections showing: a: negative immunoreaction for CD34 in group I. b, c: group II (day 26 & 31), positive cytoplasmic immune-reactivity for CD34 in the oval cells (arrow) at the periphery of the hepatic lobule around the portal tract can be detected. Group II (day 21 & 36), shows negative immunoreaction for CK 19 (not included in the figure). d, e & f: group III (day 26, 31 & 36), shows positive cytoplasmic immunoreactivity for CD34 in the oval cells (arrow) at the periphery of the hepatic lobule around the portal tract. Group III (day 21), shows negative immunoreaction for CD34 (not included in the figure). [CD34 immune-staining, x400]

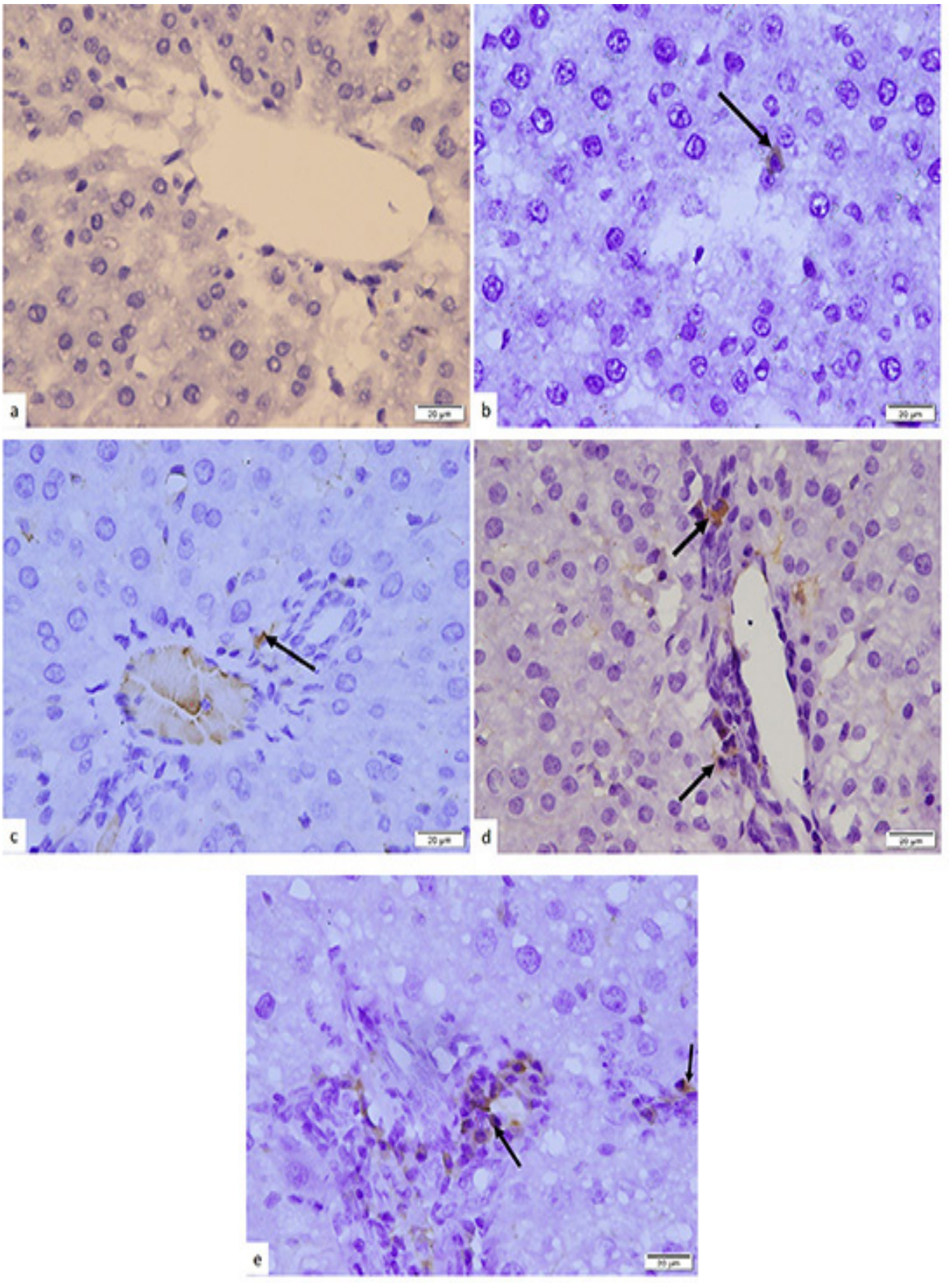

Fig. 4: Photomicrographs of liver sections showing: a: negative immunoreaction for insulin in group I. b: group II (day 31), positive cytoplasmic immune-reactivity for insulin in the oval cells (arrow) at the periphery of the hepatic lobule around the portal tract can be detected. Group II (day 21, 26 & 36), shows negative immunoreaction for CK 19 (not included in the figure). c, d, e: group III (day 21, 26 & 31), shows positive cytoplasmic immunoreactivity for insulin in the oval cells (arrow) at the periphery of the hepatic lobule around the portal tract. Group III (day 36), shows negative immunoreaction for insulin (not included in the figure). [Anti-insulin immune-staining, x400]

Table 1: Comparison between the body weights in grams (±SD) in the studied groups over the duration of the experiment:

	Mean weight (g) of Group I	Mean weight (g) of Group II	Mean weight of (g) Group III
At the start of the experiment	252.3333 ±6.11010	252.0000 ±4.84768	254.2000 ±5.16720
Day 16 of the experiment	282.3333 ±5.03322*	272.8000 ±2.86356=	274.2000 ±4.08656=
Day 21 of the experiment	289.3333 ±1.52753*	261.2000 ±2.86356=^	265.6000 ±2.40832=+
Day 26 of the experiment	295.3333 ±2.51661*	251.0000 ±3.16228=^	258.0000 ±1.58114=8+
Day 31 of the experiment	297.0000 ±2.00000*	239.0000 ±3.16228=^	249.6000 ±2.40832=8+
Day 36 of the experiment	299.0000 ±1.00000*	222.0000 ±4.12311=^	244.4000 ±2.40832=8+

*significant increase within group I
= significant decrease versus group I
8 significant increase versus group II
^ significant decrease within group II
+ significant decrease within group III

Table 2: Comparison between the blood glucose levels in mg/dl (±SD) in the studied groups over the duration of the experiment:

	Mean RBS (mg/dl) of Group I	Mean RBS (mg/dl) of Group II	Mean RBS (mg/dl) of Group III
At the start of the experiment	84.3333 ±2.08167	85.2000 ±2.77489	84.4000 ±2.40832
Day 16 of the experiment	91.6667 ±2.51661	284.4000 ±7.92465*^	285.8000 ±9.52365*+
Day 21 of the experiment	91.3333 ±1.52753	328.4000 ±4.87852*^	308.4000 ±6.65582*8+
Day 26 of the experiment	89.6667 ±1.52753	352.2000 ±4.81664*^^	332.0000 ±6.96419*8+
Day 31 of the experiment	90.3333 ±1.52753	395.0000 ±5.00000*^	308.8000 ±4.96991*8=
Day 36 of the experiment	91.5000 ±1.80278	425.2000 ±5.11859*^	268.2000 ±9.311288*8=

*significant increase versus control
8 significant decrease versus group II
^ significant gradual increase with group II
+ significant gradual increase within group III
= significant gradual decrease within group III

Table 3: Comparison between the morphometric results in the studied groups over the duration of the experiment:

	Mean area % of CK19		Mean optical density of CD34		Mean area % of insulin	
	Group II	Group III	Group II	Group III	Group II	Group III
Day 21 of the experiment	4.4530 ±.06848	9.2630 ±.19816*	.0000 ±.00000	.0000 ±.00000	.0000 ±.00000	.0000 ±.00000
Day 26 of the experiment	8.5530 ±.07689	12.1130 ±.15621*	.1940 ±10575	.6860 ±.03225*	.0000 ±.00000	2.8610 ±.08452*
Day 31 of the experiment	5.4910 ±.04228	14.7610 ±.12547*	.6180 ±.02875	1.0660 ±.02875*	.9020 ±.30535	3.4330 ±.06447*
Day 36 of the experiment	.0000 ±.00000	15.9170 ±.11767*	.0000 ±.00000	.0000 ±.00000	.0000 ±.00000	.0000 ±.00000

*significant increase versus group II

DISCUSSION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs[15].

Recently, attention has long centered on the liver in diabetes mellitus because of the importance of this organ in carbohydrate metabolism and regulation of blood sugar. The liver of all diabetic patients reveled the occurrence of hepatic changes. Hepatic oval cells appear and expand in the liver when hepatocyte proliferation is compromised. Many different markers have been attributed to these HOC, but their nature in insulin production still remain obscure.

Type 2 diabetes was chosen in this work as it accounts for more than 90% of patients with diabetes and leads to microvascular and macrovascular complications in most of the body organs. Despite increasing knowledge regarding risk factors for type 2 diabetes and evidence for successful prevention programs, the incidence and prevalence of the disease and its complications continue to rise globally[16].

This study was concerned with the hepatic progenitor (HPCs) /hepatic oval cells (HOC). They constitute 1–3% of normal liver cell pool, located mainly in the portal and peri-portal areas. They are not morphologically easy to recognize using routine histological diagnostics. Their identification with immune-histochemical markers (c-kit, CD34, Ov6, CK7, CK19, chromogranin A, CD56) also remains particularly difficult as these cells are very few in number and most of the markers are not specific[17,18,19].

In the current study; gradual decrease in body weight in group II (diabetic group) and group III (treatment group) was recorded, this was in co-ordinance with a previous study[20] which stated that weight loss may take the form of muscle wasting and loss of muscular strength. Weight loss may also be accompanied by extreme, constant hunger (polyphagia) as the body lacks the ability to use glucose. This also come in line with another former study[21] which reported that adults with either diagnosed or undiagnosed type 2 diabetes showed excessive loss of trunk fat mass compared with non-diabetic subjects. Such weight loss is considered as one of the diabetic symptoms[15].

The rate of body weight loss was slower in group III when compared to group II. This might be based on that vitamin K2 could increase the sensitivity of the body cells to insulin reducing body weight loss[22]. Such insulin sensitivity improvement was proved recently to be due to vitamin K2 anti-inflammatory and lipid-lowering properties[23].

Concerning measuring the random blood sugar level, the current study recorded that the diabetic group showed hyperglycemia among 70% of the animals while the treated group showed it in only 40% of the experimental animals. This finding is concomitant with that of a recent study[24] which demonstrated that circulating vitamin K2 had a positive effect on lowering fasting glucose level. This might be explained through that administration of vitamin K2 may help insulin sensitivity of the cells[23,24,25].

Examination of the prepared histological sections from group II &III revealed that hepatocytes around the central vein and near the portal tract showed rarefaction & vacuolation of the cytoplasm, pyknotic nuclei and apoptotic changes. This phenomenon was stated previously[26] based on that diabetes mellitus is the most common cause of progressive hepatic fatty steatosis, hepatic changes, hydropic changes and cloudy swellings observed in the hepatocytes. It was explained by the liver mitochondrial dysfunction in diabetes with subsequent oxidative stress. In another study[27] diabetic degenerative changes were reported to include vacuolations of the hepatocytes in the central zonal area of the hepatic lobule and disruption of the entire liver architecture.

Group III of the current study showed less rarefaction & vacuolations of the hepatocytes. This observation could be suggested to be due to vitamin K2 treatment where it was proved to cause oval cell expansion that enhanced liver regeneration after partial hepatectomy[8].

In the current study, we demonstrated great number of MNCI in the portal tract and around the central vein areas with congestion of the blood sinusoids and the branches of the portal veins. Such result was presumed to result from hyperglycemia induced oxidative stress in the liver tissue which is characterized by de-arrangement of protein, carbohydrates and lipid metabolism. This pathological condition in liver of the diabetic patients might progress to hepatic inflammation with lymphocytic, neutrophilic and other inflammatory infiltrates, hepatic necrosis and fibrosis known as non-alcoholic steatohepatitis (NASH)[27]. In another study[26], it was reported that STZ induced several morphological and histological alterations in the liver tissues that included lipid deposition, inflammatory cells infiltration and Kupffer cells hyperplasia. Moreover, one of the typical characteristic changes observed in diabetic liver diseases in a recent study[28] was the appearance of mixed inflammatory cells infiltrating the portal area which include mononuclear cells and few neutrophils.

In the present study, examination of the CD34 marker immune stained sections (as a stem cell marker) demonstrated positive cytoplasmic reaction in cells at the center and periphery of the classic hepatic lobule. Bearing in mind that diabetic liver injury leads to oxidative stress changes accompanied by the production of cytokines and inhibition of hepatocytes proliferation[28]. Additionally, HOC are a small subpopulation of hepatic cells that believed to be bi-potentialable to differentiate into hepatocytes or bile ductular cells in case of depressed hepatocytic proliferation[29]. Hence, the CD34 positive cells detected in this work could be suggested to be oval cells. Further support to this suggestion was the identification of CK19 (oval cell marker) positive cells at the center and periphery of the classic hepatic lobule in the

experimental rats. This was in co-ordinance with previous studies[30,31] which reported that although adult hepatic stem cells known as HOC were scarcely detectable under physiological conditions and during the normal process of liver regeneration, presumably because of their small numbers, they could proliferate and differentiate into both the hepatic and cholo-angiocytic lineages in cases with liver disease. Furthermore, oval cells expansion was reported to occur following partial hepatectomy as a result of the associated hepatic inflammatory infiltration[32], which was already present in the current study caused by diabetes.

In the current study examination of the anti-insulin marker (for insulin production) immune stained sections demonstrated positive reaction in some cells of MNCI in the treatment group on day 26 & 31 of the experiment and in the diabetic group on day 31 of the experiment. These results were previously explained[33,34] by the trans-differentiation of the hepatic oval cell population to insulin producing β-cells. This in turn resulted in amelioration of hyperglycemia and increase in insulin content after STZ treatment. Such trans-differentiation could be explained by the common developmental origin of liver and pancreas[31].

The significant increase in mean optical density of CD34 and mean area present of CK19 in addition to the early and sustained production of insulin in the treated group versus the diabetic group might be explained by vitamin K2 administration. This explanation was based on that vitamin K2 had a great role in hepatic injury accompanying diabetes where it promotes liver functional recovery and regeneration through increasing the life span of oval cells[8].

CONCLUSION

Hepatic oval cells may be differentiated to insulin producing cells in cases of diabetes thus acting as an extra pancreatic source of insulin. Moreover, vitamin K2 may help and promote the function of HOC through increasing their life span in case of diabetes.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

دراسه هستولوجيه و هستوكيميائية مناعيه علي التأثير المحتمل لفيتامين ك٢ علي الخلايا البيضاويه الكبديه في الجرذان البيضاء المصابه و الغير مصابه بداء السكري

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الخلفية: مرض السكري هو مجموعة من الأمراض الأيضية التي تتميز بارتفاع سكر الدم الناتج عن نقص هرمون الأنسولين ، أو عمل الأنسولين المعيب ، أو كليهما. تجديد الخلايا β من الخلايا الجذعية أو الخلايا السلفية للبنكرياس هو طريقة جذابة لعلاج مرض السكري. يمكن للخلايا البيضاوية الكبدية (HOCs) ، خلايا داخل الكبد لها قدرات سلفية ثنائية قوية ، أن تتكاثر وتتمايز إلى كلا من الخلايا الكبدية والخلايا الظهارية في القناة الصفراوية. و لديهم القدرة على التحول إلى خلايا الغدد الصماء للبنكرياس ، لأن كلا من الكبد والبنكرياس لديهم نفس المنشأ (الأديم الباطن العلوي الأمامي البعيدة). يمكن أن يعزز فيتامين ك٢ الشفاء لدى المرضى الذين يعانون من تليف الكبد ويمنع مقاومة الأنسولين مما يقلل من خطر الإصابة بالسكري من النوع ٢.

الهدف من العمل: تقييم التغيرات النسيجية والهستوكيميائيةالمناعية في الخلايا الكبدية والخلايا البيضاوية في كبد الجرذان المصابة والغير مصابة بداء السكري تحت العلاج بفيتامين ك٢.

المواد وطرق البحث: تم تقسيم ٨٨ من ذكور الجرذان البيضاء إلى ٣ مجموعات: المجموعة الأولى (مجموعة التحكم) ، المجموعة الثانية (مجموعة السكري) ؛ حيث تم إحداث داء السكري باستخدام جرعة واحدة في الغشاء البريتوني(IP) من الستربتوزوتوسين (STZ) بعد أسبوعين من اتباع نظام غذائي غني بالدهون ومجموعة III (مجموعة العلاج) حيث تم إعطاء جرذان مصابة بداء السكري يوميًا عن طريق الفم فيتامين ك٢ لمدة ٢٠ يومًا (٥ أيام و ٥ أيام خارج).ثم تم قياس نسبة السكر العشوائية في الدم لكل مجموعة في اليوم ٠ و ١٦ ثم كل ٥ أيام حتى نهاية التجربة (٣٦ يوما). وقد صبغت جميع عينات هذه المجموعات بصبغة H&E و الصبغة الهستوكيميائية المناعية للـ CD34 و CK19 والأنسولين. تم قياس الكثافة الضوئية للتفاعل الإيجابي لل CD34 ومتوسط النسبة المئوية لمساحة التفاعل المناعى لل CK19 والأنسولين ، وتم تحليل البيانات إحصائيا.

النتائج و الخلاصة: أعطت HOCs رد فعل إيجابي حيث تغيرت الى الخلايا المنتجة للأنسولين في الجرذان المصابة بداء السكري وبالتالي يمكن تعديلها لتتغير إلى مصدر للأنسولين خارج البنكرياس. بالإضافة إلى ذلك ، ساعد تناول فيتامين ك٢ على زيادة القدرة التكاثرية لـ HOCs في حالة مرض السكري.