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ORIGINAL PAPER

Effects of diabetes mellitus on the rat liver during the postmenopausal period

Deniz Unal · Selina Aksak · Zekai Halici · Ozlem Sengul · Beyzagul Polat · Bunyami Unal · Mesut Halici

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Abstract The present study investigated whether diabetes worsened the onset of liver injury/damage during the ovariectomized (OVX)-induced postmenopausal period in rats. Diabetes results in severe complications in humans, such as liver failure. Estrogen and its derivatives are medically acceptable, powerful antioxidant agents that can enable liver and other important organs to defend themselves against oxidative related injury. Estrogen deficiency, which occurs in the postmenopausal period and in individuals with diabetes, may play a significant role in the progression of liver failure. In the present study, rats were divided into four groups: control (Group I), diabetic (Group II), ovariectomy (Group III) and ovariectomy plus diabetes (Group IV). After the experiments, quantitative histopathological and immunohistochemical changes in liver were detected using light microscopy and modern stereological systems. Histopathological examinations showed that there were many necrotic and apoptotic hepatocytes in the lobules of Group II. In addition, there were a larger number of necrotic cells in Group III than Group II. In contrast to Group II, there were also apoptotic cells in the portal areas in Group III. Moreover, evidence of liver injury was higher in the sections of Group IV compared with all other groups. In biochemical findings, there were statistically significant differences between all the groups ($P < 0.001$) for catalase (CAT), glutathione peroxidase (GSH) and myeloperoxidase (MPx) activity. In addition, the amount of lipid peroxidation (LPO) was significantly different between groups. In stereological results, there were significant differences between Groups I and II and Groups II and IV. The present study provided novel insight into the pernicious effects of ovariectomy on liver injury following the onset of diabetes. Indeed, the present study found that increases in liver oxidative activity in OVX rats following the onset of diabetes correlates with elevated MPx, LPO and histopathological changes in rat liver.

Keywords Aging · Diabetes · Oxidative stress · Rat · Liver · Stereology

Introduction

The liver, which plays a critical role in lipid, carbohydrate and protein metabolism, performs tasks such as bile manufacture, vitamin storage, and the detoxification of drugs and toxins. In addition, the liver plays a role in immune functions. The liver is the largest gland in the body, and it has both endocrine and exocrine functions. Abnormalities may arise in any situation where the normal liver architecture, which is constituted of parenchymal and stromal components, is affected and/or disrupted. One of these abnormalities is liver failure, which is a complex syndrome characterized by the impairment of many different organs and body functions. In the liver, pathological conditions affecting the parenchyma or the stroma are not always

D. Unal · S. Aksak · B. Unal
Department of Histology and Embryology, Ataturk University, Faculty of Medicine, 25240 Erzurum, Turkey

Z. Halici (✉) · B. Polat
Department of Pharmacology, Ataturk University, Faculty of Medicine, 25240 Erzurum, Turkey
e-mail: hzekai@atauni.edu.tr

O. Sengul
Department of Gynecology and Obstetrics, Yenimahalle Government Hospital, Yenimahalle, Ankara, Turkey

M. Halici
Department of Biochemistry, Ataturk University, Faculty of Veterinary, 25240 Erzurum, Turkey

restricted to a particular area or structure. Destructive effects may appear not only in the liver but also in many different organs, which can also effect the functions associated with those organs (Schmucker 2005).

Diabetes, which is an important endocrine/metabolic health problem, causes many complications in a variety of organs. In addition, menopause, which is a biological process that occurs as part of aging in women, leads to a series of related troubles. Both conditions generate oxidative stress via different mechanisms in the liver and lead to negative effects (Cavadas et al. 2010).

Recent evidence has indicated that there is increased oxidative damage in diabetes mellitus, which occurs through different mechanisms. For example, studies have suggested that hyperglycemia can increase oxidative stress by generating an excess of mitochondrial nicotinamide adenine dinucleotide (mNADH) and reactive oxygen species (ROS) and disrupting the balance between caloric intake and energy consumption, which changes the redox potential of glutathione (Maddux et al. 2001; West 2000).

Many studies have shown that estrogen is a critical hormone for the regulation of oxidative stress, serum lipid concentrations, coagulation and fibrinolytic systems, antioxidant systems and the production of other vasoactive molecules, such as nitric oxide and prostaglandins. All of these effects can influence the development of vascular disease (Mendelsohn and Karas 1999). The average menopause age in women is 51 years, and menopause causes dramatic hormonal changes, such as the ablation of estrogen, that can affect the immune-regulatory system. Estrogens, which are female sex hormones, regulate growth, differentiation, and the function of many reproductive tissues. Estrogens also affect other important tissues, such as heart, blood vessels, bone, liver, and some brain cells. As women undergo menopause, estrogen concentrations in the blood decrease, which causes physiological changes, including an increased level of ROS, that have been shown to increase the risk for some diseases (Bernardi et al. 2003; Stevenson et al. 2005). Hormonal stability is one of the major factors necessary for safeguarding the reproductive functions of living organisms because hormonal instability may disturb metabolic processes. Estrogen and its derivatives are medically accepted, powerful antioxidant agents that enable liver and other important organs to defend themselves against oxidative-related injury (Shimizu 2003). Ovariectomy (OVX) surgery in rats stimulates menopause (Vom Saal et al. 1994). In addition, OVX caused decreased glutathione S-transferase (GST) activity in the cytosol and microsomal fractions and increased mitochondrial oxidative damage in liver and renal tissue. Interestingly, the replacement of female sex hormones, such as estrogen and progesterone, can improve lipid peroxidation by activating the antioxidant system (Kireev et al. 2007; Oztekin et al. 2007a, b). Indeed, Kumru et al. 2005 demonstrated that estrogen replacement therapy in postmenopausal woman ameliorated high levels of plasma malondialdehyde (MDA). In addition, Moreira et al. 2007 showed that although estrogen strongly protects against lipid peroxidation, its protection profoundly affects liver mitochondrial function.

In animals with diabetes and sepsis, we have previously shown that OVX-induced estrogen deficiency results in general metabolic changes in liver, lungs, heart and kidney (Uyanik et al. 2010; Albayrak et al. 2009). In addition, Kireev et al. demonstrated that ovariectomized old rats produced significantly increased levels of the pro-inflammatory cytokines, whereas the anti-inflammatory cytokine IL-10 decreased in liver. In the same study, Kireev et al. 2010 showed that the administration of estradiol was accompanied by decreased liver inflammation in ovariectomized female rats.

The present study was designed to investigate whether diabetes worsened the onset of liver injury/damage during the OVX-induced postmenopausal period in rats. We examined the effects of menopause and diabetes on the livers of rats separately and together using three distinct methods: histopathological detection with the help of a light microscope, quantitative analyses by means of stereological tools and a biochemical evaluation of liver tissue.

Materials and methods

Animals and experimental groups

Animals were housed in facilities accredited by international guidelines, and the studies were approved and conducted in accordance with the Institutional Animal Care and Use Committee of Ataturk University. The present study used 24 adult (12 weeks old) female Sprague–Dawley rats from Ataturk University Experimental Animal Laboratory (ATADEM). The animals were housed in groups of six per cage for at least 7 days under controlled conditions of constant temperature/humidity, and the rats were exposed to a 12-h light/dark cycle.

Twenty-four, twelve-week-old female Sprague–Dawley rats were randomly allocated into four groups: (i) nondiabetic healthy control group (Group I, $n = 6$), (ii) diabetic group (Group II, $n = 6$), (iii) OVX group (Group III, $n = 6$), and (iv) OVX plus diabetes group (Group IV, $n = 6$) (Table 1).

Experimental models

Ovariectomy procedure

Bilateral ovariectomy was performed by making a longitudinal incision (0.5–1 cm) in the midline area of the lower

Table 1 All details of experimental protocol

Groups	1. Day of experiment	1–90 Days of experiment	91. Day of experiment	91–122 Days of experiment	123. Day of experiment
Group 1 (N:6) non-diabetic healthy control group	Waiting period				End of experiment
Group-II (N:6) diabetic group	Waiting period		Diabetes induction with alloxan	Waiting period	
Group-III (N:6) ovariectomy group	Ovariectomy operation	Waiting period	Waiting period	Waiting period	
Group-IV (N:6) ovariectomy plus diabetic group	Ovariectomy operation	Waiting period	Diabetes induction with alloxan	Waiting period	

abdomen, removing the ovaries and closing the skin incision (Albayrak et al. 2009; Kharode et al. 2008). After ovariectomy, rats were given 25 mg/kg metamizol sodium as an analgesicfor 2 days. Ovariectomized rats were kept alive for 12 weeks. After 12 weeks, diabetes was induced in two groups of rats (one group of ovariectomized rats and one group of nonovariectomized rats).

Alloxan-induced diabetes procedure

Diabetes was induced in female Sprague–Dawley rats by intraperitoneal administration of aqueous alloxan monohydrate (a single dose of 150 mg/kg body weight, Sigma–Aldrich Co, Germany) according to previously described methods (Halici et al. 2009). Alloxan was freshly prepared in 0.9% NaCl solution and injected intraperitoneally to rats that were fasted for one night. After alloxan application, the pancreas secretes insulin at high levels, which can cause fatal hypoglycemia. To prevent this adverse effect, 5 ml of 20% glucose solution was injected intraperitoneally 4–6 h after alloxan, and a 5% glucose solution was added to the drinking water for 24 h and food intake was allowed. 72 h after alloxan administration, blood samples were taken from the tail vein of the rats to determine fasting blood glucose levels in plasma by an Accu-Chek Active® blood glucose monitor. A diabetic rat was defined as having a serum glucose level of at least 200 mg/dl, and diabetic rats were kept alive for 8 weeks.

Research methods

Histological examination

Dissection and histological examination in paraffin sections Each liver was fixed in 10% formalin solution for 48–55 h, dehydrated in a graded alcohol series, embedded in paraffin wax, and serially sectioned using a microtome (Leica RM2125RT). Serial 40-μm sections were mounted onto glass slides for stereological analyses. To estimate the number of hepatocytes, selected sections were stained with hematoxylin and eosin. For light microscope histological examination, thin 5-μm sections were taken from the same paraffin blocks. Sections were stained with hematoxylin and eosin. The slides were covered, and photographs were taken using a light microscope with a camera attachment (Nikon Eclipse E600, Japan).

Immunohistochemistry by TUNEL in paraffin sections To detect DNA breaks, in situ cell death detection kits for the TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) method were purchased from Roche Applied Science (Penzberg, Germany). The sections were deparaffinized and treated with proteinase K solution (20 μg/ml in PBS) for 15 min at room temperature. Subsequently, the sections were washed in distilled water and immersed in 3% hydrogen peroxide for 15 min. After several washes in PBS (50 mM sodium phosphate and 200 mM NaCl at pH 7.4), the sections were immersed in equilibration buffer at room temperature for 20 min. The sections were then incubated with terminal deoxynucleotidyl transferase (TdT) enzyme at 37°C for 1 h in a humidified chamber, and the reaction was stopped by immersion in a stop/wash buffer. After several washes, the sections were incubated in antidigoxigenin-peroxidase for 30 min at room temperature. The reaction was revealed with 0.06% 3,3-diaminobenzidine tetrahydrochloride (Sigma Chemical, St. Louis, MO) in PBS for 3–6 min, and the sections were counterstained with Mayer's hematoxylin. The sections were examined and photographed under a light microscope (Olympus BH-40) (Altunkaynak et al. 2009).

Biochemical investigation

Biochemical investigation in liver tissues Rat livers were kept at −80°C for 3 days for biochemical investigation, and catalase (CAT), superoxide dismutase (SOD) and myeloperoxidase (MPO) activities and the amount of glutathione (GSH) and lipid peroxidation (LPO) were determined in rat liver tissues.

To prepare the tissue homogenates, tissues were ground in a mortar with liquid nitrogen. The ground tissues (0.5 g

each) were mixed with 4.5 ml of the appropriate buffer, and the mixtures were homogenized on ice using an Ultra-Turrax homogenizer for 15 min. The homogenates were filtered and centrifuged using a refrigerated centrifuge at 4°C, and the supernatants were used to determine enzymatic activities and amounts. All assays were performed at room temperature in triplicate (Cadirci et al. 2010a, b; Karakus et al. 2009).

Catalase, SOD, and MPO activities and GSH and LPO levels were determined according to the methods of Aebi (1984), Sun et al. (1988), Bradley et al. (1982), Sedlak and Lindsay's (1968) and Ohkawa et al. (1979), respectively (Aebi 1984; Bradley et al. 1982; Ohkawa et al. 1979; Sedlak and Lindsay 1968; Sun et al. 1988).

Quantitative analyses

Stereological estimation All sections were obtained from each block (without sampling procedures) for stereological analyses. The number of hepatocytes was estimated with the optic dissector method using Stereoinvestigator software (Microbrightfield, CA, USA). The equipment was composed of a charge-coupled device digital camera (Optronics MicroFire), a personal computer and computer-controlled motorized specimen stage (Bio- Precision MAC 5000 controller system), and a light microscope (Leica DM4000 B). Each hepatocyte was counted according to the unbiased counting rules of the optical dissector (Sterio 1984).

According to a pilot study, a 3,062,500-μm^2 (in X, 1.75 mm; in Y, 1.75 mm) step size was detected for microscopic sampling, which was suitable for performing stereological analysis in our study. We used an unbiased counting frame (0.065×0.065 mm = 4,225.00 μm^2) in all steps.

According to the optic dissector counting rules, each dissector probe that gives rise to a three-dimensional (3D) counting box has to have a lower height than the section thickness. The height of the dissector probe was 16 μm, and the thickness of the sampling fraction was 26–29 μm. All hepatocytes were counted in each sampled dissector probe during stereological analysis (Gray 1996; Halici et al. 2009; Uyanik et al. 2009).

Results

Histopathological results

Conventional light microscopy by hematoxylin and eosin staining

We evaluated two parts of each section: the classic liver lobule, including the middle of the central vein, the hepatic cells that radiate out from the central vein and sinusoids between hepatocyte cords; and the portal area, which consists of hepatic arteriole branches, portal veins, bile ducts, and connective tissue surrounded by hepatocytes.

Healthy control group When we evaluated the control group, the central vein was observed in terms of both its regular line (Fig. 1a, b, e, f) and its endothelial cells with native features (Fig. 1b, e). In addition, the hepatocyte cytoplasms were stained a pink color by eosin (Fig. 1a–c), and cytoplasms contained basophilic granules (Fig. 1d–f). Some hepatocytes had two nuclei (Fig. 1e, f), and some nuclei had two or more nucleoli (Fig. 1b, d–f).Sinusoids between cords had natural width, and they were lined with endothelial cells in some places (Fig. 1a, b, e, f). Bile duct cells and hepatic arterioles with endothelial cells that protruded into the lumen were considered to be normal. Hepatocytes close to this area had more basophilic cytoplasm than those located near the central vein.

Diabetic group In the diabetic group, degeneration of hepatocytes surrounding the central vein was conspicuous at first glance (Fig. 2a, b). Indeed, the observed hepatocyte degeneration involved irregularity in the cell range, transparency in the cytoplasm and pyknosis and hyperchromasia in the nuclei. In addition, sinusoidal dilatation (Fig. 2d) was observed in section profiles, which was not observed in the control group. Moreover, there was a significant narrowing of sinusoids in the portal areas (Figs. 2c, e, 3a, c, e). In addition, portal hepatocytes were irregularly shaped, particularly in terms of the surfaces of adjacent hepatocytes (Fig. 3a–c, e). Furthermore, there was damage in the endothelial cells of portal veins (Fig. 3a, d). We also observed increasing amounts of connective tissue and inflammatory cells in the portal area (Figs. 2c, e, 3d, e).

Ovariectomy group In the OVX group, liver cords were irregularly shaped, and sinusoids were narrow (Fig. 4a–e). In addition many inflammatory cells were observed around the central vein and sinusoids (Fig. 5c). Hepatocytes close to both the central vein and sinusoidal endothelial cells (Fig. 4d, e) exhibited hyperchromatic nuclei. In the same fields, we found irregularly shaped hepatocytes that had lost their connection to other hepatocytes (Figs. 4e, d, 5d, e).

Similar to the diabetic group, significant damage was apparent in the endothelial cells of portal veins in the OVX group (Figs. 4a, b, 5a). In addition, sections from the OVX group showed conspicuous endothelial damage in the arterioles (Figs. 4a, 5a).

Ovariectomy plus diabetes group In the OVX plus diabetes group, liver damage was more severe than the other

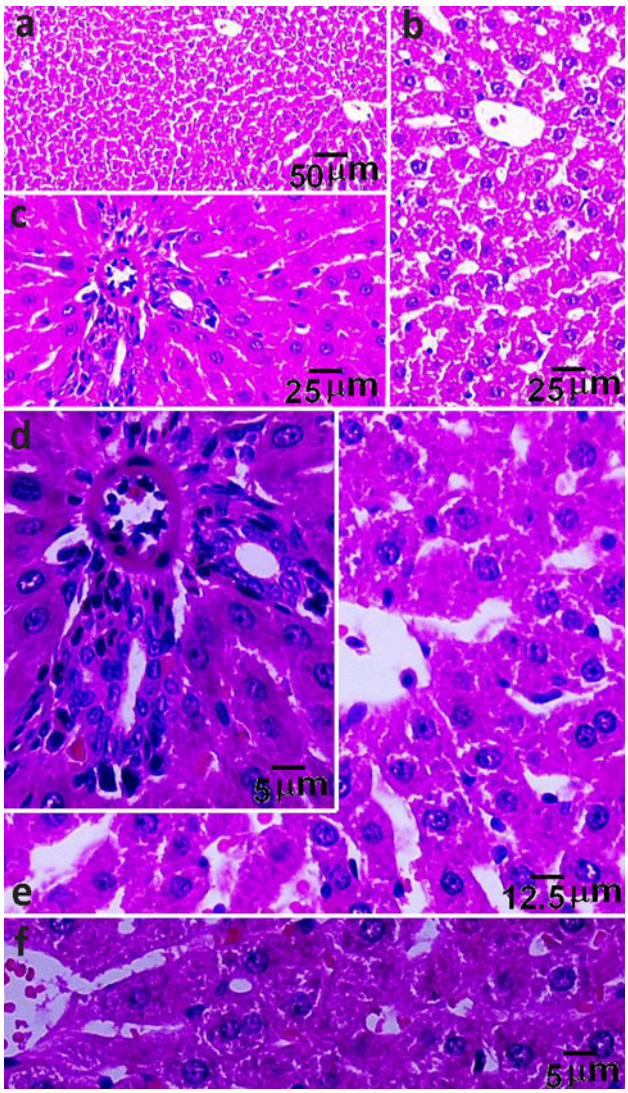

Fig. 1 Light microscopic photomicrograph of healthy control groups (Stain: Hemotoxylen Eosin). Sections were showed at different magnification and different areas. **a**, **b**, **e**, **f** central vein, hepatocytes that radiate out from the central vein and sinusoids. **c**, **d** portal area and peri portal structures

groups. Total obstruction was apparent in both the central veins (Figs. 6c, 7a, d) and hepatic arterioles (Figs. 6d, 7b, e). The nuclei of almost all hepatocytes around the central vein were hyperchromatic (Fig. 6a, c). In many of the periportal areas, we identified hepatocytes with hyperchromatic nuclei (Figs. 6b, d, e, 7b–d) and small necrotic foci consisting of necrotic cells (Fig. 6b, e).

Immunohistochemistry by TUNEL

Healthy control group TUNEL staining did not reveal any abnormalities in the control group (Fig. 8).

Diabetic group In the diabetic group, we observed many immunoreactive hepatocytes in lobules (Fig. 9a–d); however, there were fewer immunoreactive cells in portal areas (Fig. 9g, h).

Ovariectomy group In the OVX group, intense immunoreactive hepatocytes were found in the lobules (Fig. 10a–d) and in portal areas (Fig. 10f).

Ovariectomy plus diabetes group In the sections from the OVX plus diabetes group, intense nuclear positivity was observed in most of the hepatocytes (Fig. 12a–c). Interestingly, immunoreactive cells were localizedaround the central vein (Figs. 11a, 12e) and bile ducts in the portal areas (Figs. 11b–d, 12a–d, f).

Biochemical results

The present study examined LPO levels as an indicator of oxidative stress. In addition, we measured CAT and SOD enzyme activities and GSH levels to understand the behavior of defense mechanisms in the liver. In addition, MPx activity was studied as an indicator of neutrophil infiltration, which is a marker of inflammation. The results are presented in Figs. 13, 14, 15.

In the diabetic, OVX, and OVX plus diabetes groups, there was a progressive increase in CAT activity compared with the control group ($P < 0.05$). In addition, there were significant differences in CAT activity between the experimental groups ($P < 0.05$) (Fig. 13). Compared with the control group, the mean percent increase in the diabetic, OVX and OVX plus diabetes groups were 18.4, 40.2 and 61.4%, respectively.

We also observed significant differences in SOD activity between the control group and both the diabetes and OVX groups ($P < 0.05$); however, there was no difference between the control group and the OVX plus diabetes group ($P > 0.05$). In addition, there were significant differences between the experimental groups ($P < 0.05$) (Fig. 13). Compared with the control group, the mean percent change in the diabetic, OVX and OVX plus diabetes groups were 19.9% (−), 13.8% (+) and 3.1% (−), respectively.

The MPx activity was significantly different in all experimental groups compared with the control group ($P < 0.05$). MPx was also significantly different between the experimental groups ($P < 0.05$) (Fig. 14). Interestingly, there was a progressive increase in MPx activity in the diabetic group and an even bigger increase in the OVX and OVX plus diabetes groups. Compared with the control group, the mean percent increases in the diabetic, OVX, and OVX plus diabetes groups were 16.1, 112.5 and 179.4%, respectively.

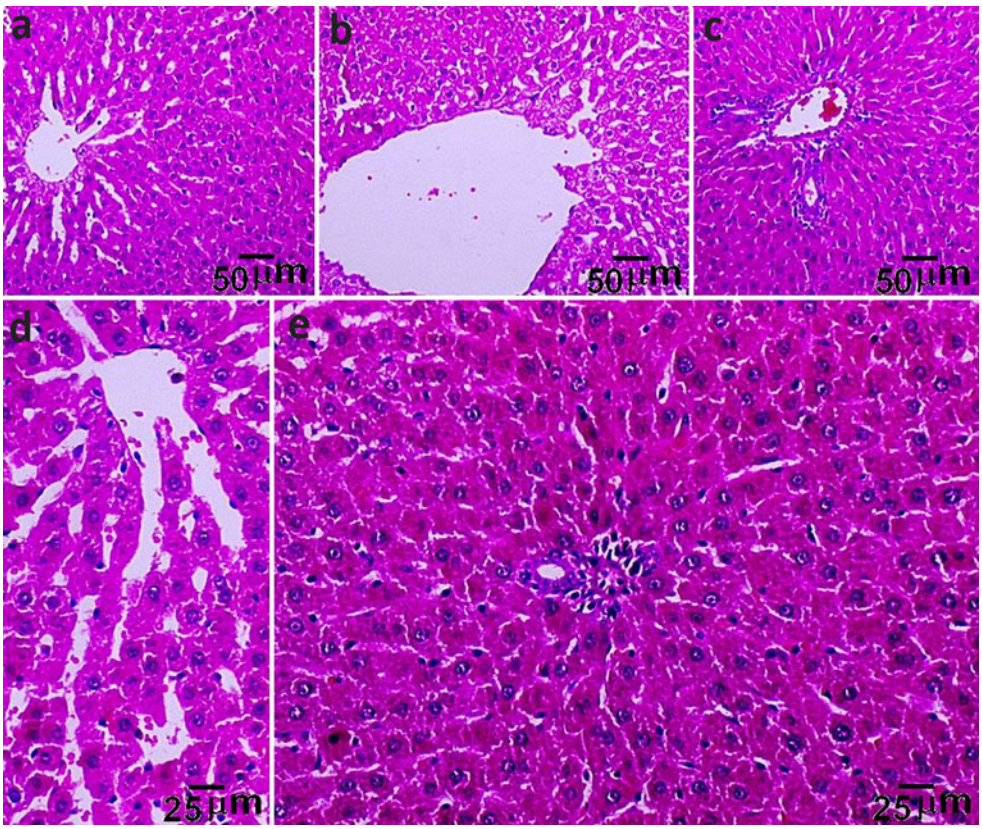

Fig. 2 Light microscopic photomicrograph of diabetic groups (Stain: Hemotoxylen Eosin). Sections were showed at different magnification and different areas. **a**, **b**, **d** central vein, dilated sinusoids and perisinusoidal hepatocytes. **c**, **e** portal area, peri portal hepatocytes had more eosinophilic cytoplasm and hyperchromatic nuclei and sinusoidal narrowing

Interestingly, the amount of LPO was statistically lower in the control group compared with the other groups ($P < 0.05$). LPO was also significantly different between the experimental groups ($P < 0.05$) (Fig. 14). Compared with the control group, the mean percent changes in the diabetic, OVX and OVX plus diabetes groups were 135.9, 86.6 and 214.6%, respectively.

There was also a significant difference in the amount of GSH between the control and experimental groups ($P < 0.05$). In addition, there were significant differences in GSH levels between the experimental groups ($P < 0.05$) (Fig. 15). Compared with the control group, the percent changes of GSH in the diabetic, OVX and OVX plus diabetes groups were 5.9%(−), 11.04%(+) and 6.8%(+), respectively.

Stereological results

The values for the numerical density of hepatocytes in all groups are shown in Fig. 16. The statistical analysis of the hepatocyte densities showed that there were significant differences between the control group and the diabetic group and OVX plus diabetes group ($P < 0.05$; one-way analysis of variance [ANOVA]); however, there was not a significant difference between the control group and the OVX group.

Discussion

Although menopause and diabetes have different clinical aspects and physiopathological mechanisms and affect different basic functions, there are cellular and subcellular similarities. Indeed, both menopause and diabetes cause similar changes in serum biochemical parameters associated with particular functions, including oxidative stress enzymes, and histopathological findings in the liver.

The present study examined and evaluated the direct and indirect results of diabetes and menopause-induced liver injury. We also attempted to explain the relationship to the physiopathological events involved in the liver metabolism.

We wanted to determine what histopathological or quantitative arguments could be used to prove the findings

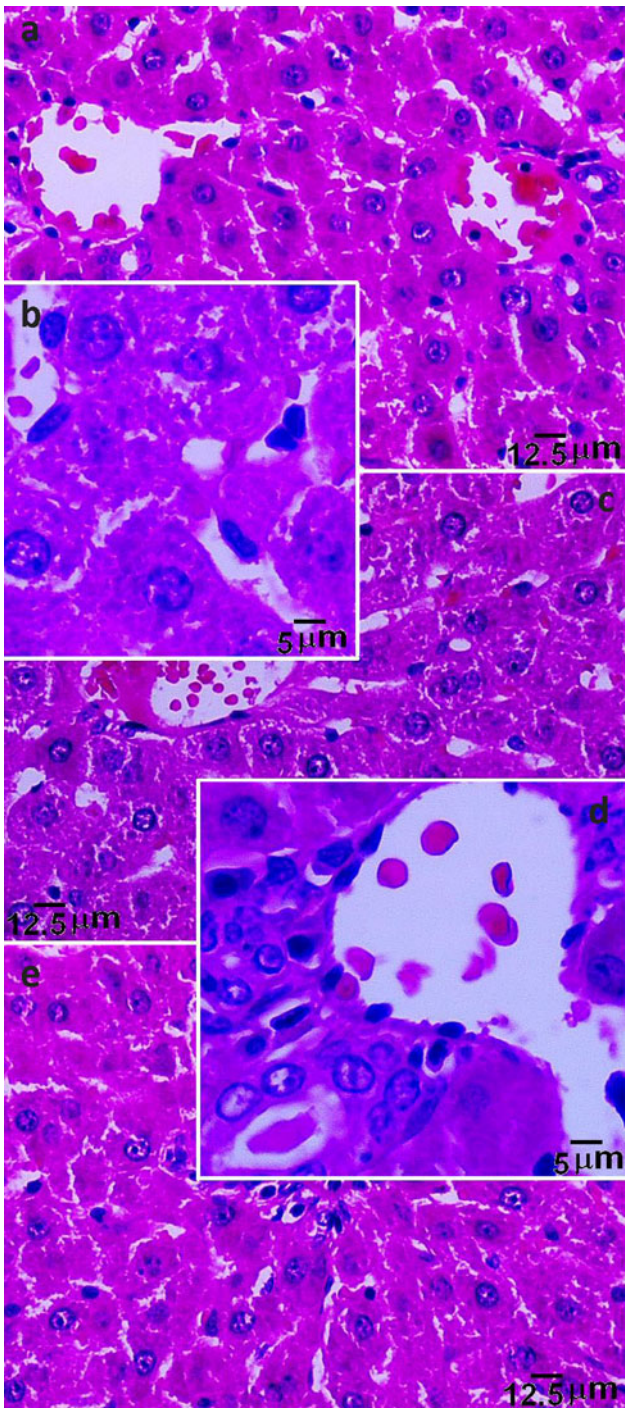

Fig. 3 Light microscopic photomicrograph of diabetic groups (Stain: Hemotoxylen Eosin). Sections were showed at different magnification and different portal areas. **a–e** damage in both portal vein and sinusodal endothelial cells, irregular shaped hepatocytes

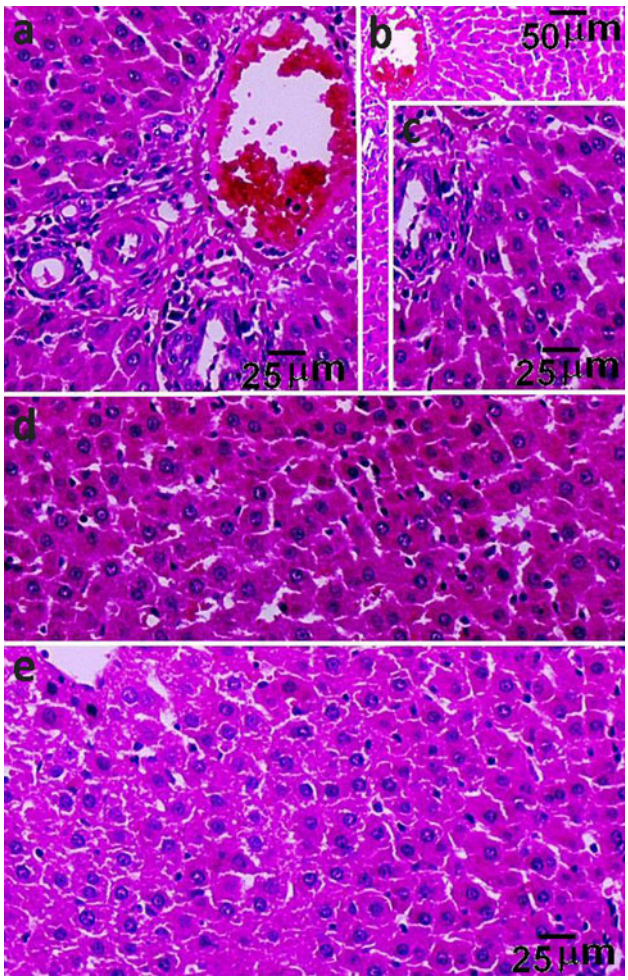

Fig. 4 Light microscopic photomicrograph of ovariectomy groups (Stain: Hemotoxylen Eosin). Sections were seen at different magnification and different areas. **a–c** portal area with damaged vessel's endothelial cells and increased inflammatory cells. **d**, **e** irregularly shaped, more eosinophilic hepatocytes

of the present study. Interestingly, a comparison of the diabetic and OVX groups showed that both apoptotic and necrotic cell ratios and the distribution pattern of apoptotic cells in various parts of liver exhibited some differences that could affect the clinical situation of patients. For example, immunopositive hepatocytes in the diabetic group were principally located in the lobule, whereas apoptotic cells in the OVX groups were primarily found in the portal areas. We also observed a greater number of necrotic cells in the hepatocytes of the OVX groups compared with the diabetic group. Indeed, the primary effect in the diabetic group was the presence of apoptosis rather than necrosis in the parenchymal components of the liver lobule, whereas the primary effect in the OVX groups were the presence of necrotic rather than apoptotic hepatocytes in the lobule. Interestingly, both necrotic and apoptotic cells were present in the portal area in the OVX group.

The numerical density results that we obtained using stereological methods were quite surprising in the diabetic group. There was a mean rise of 21% in the rate of the diabetic group. In contrast, there was a mean decrease of 6 and 7% in the OVX and OVX plus diabetes groups,

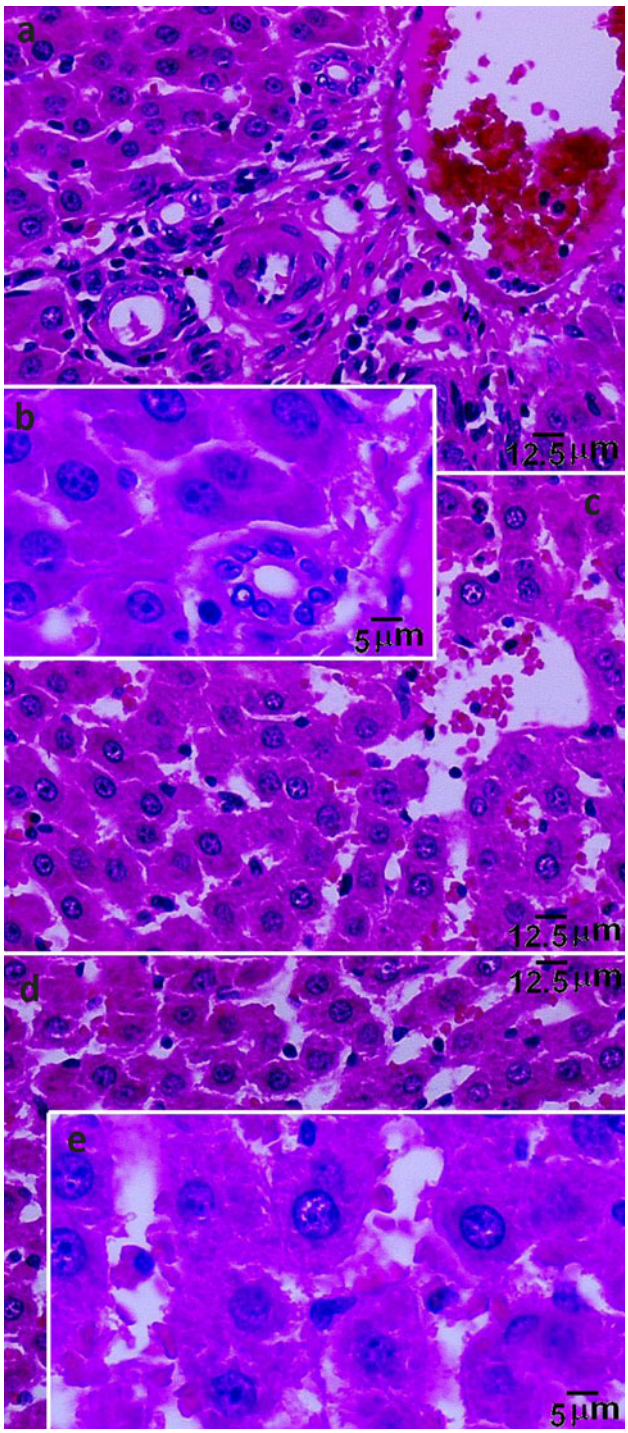

Fig. 5 Light microscopic photomicrograph of ovariectomy groups (Stain: Hemotoxylen Eosin). Sections were seen at different magnification and different areas. **a**, **b** inflammatory cells infiltration in portal area **c**–**e** intrasinusoidal inflammation

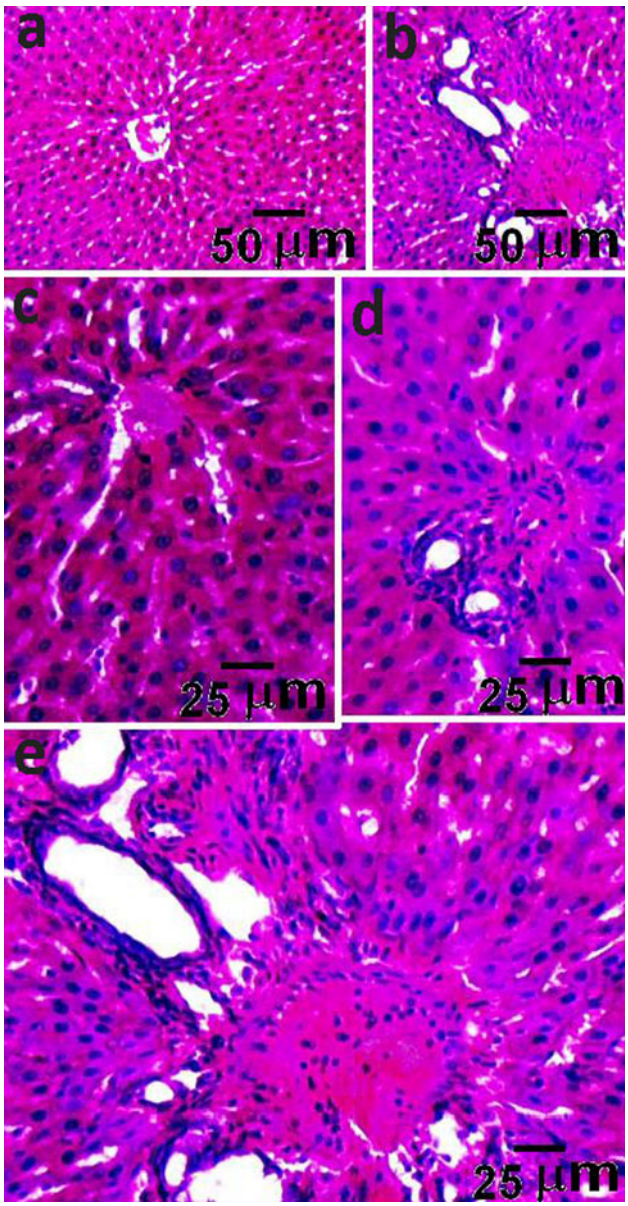

Fig. 6 Light microscopic photomicrograph of ovariectomy plus diabetic groups (Stain: Hemotoxylen Eosin). Sections were seen at different magnification and different areas. **a**, **c** central vein obstruction, **b**, **e** necrotic focuse, **d** hepatic arteriolar obstruction

respectively, compared with the control group, which was expected.

Numerous studies have found that hyperplasia occurs to compensate for insulin resistance in other structures, such as pancreatic β cells (Kulkarni et al. 2004), adipose cells (Kahn and Flier 2000) and smooth muscle cells (Ginsberg 2000). Only one study by Halici et al. (2009), however, has shown an increase in the number of hepatocytes.

Interestingly, there were more necrotic cells than apoptotic cells in OVX rats compared with the diabetic group. The findings of the present study suggested the presence of irreversible cell injury because of the degree of damage in the mitochondrial structures, and this type of damage would be expected to elicit more necrotic than apoptotic cells.

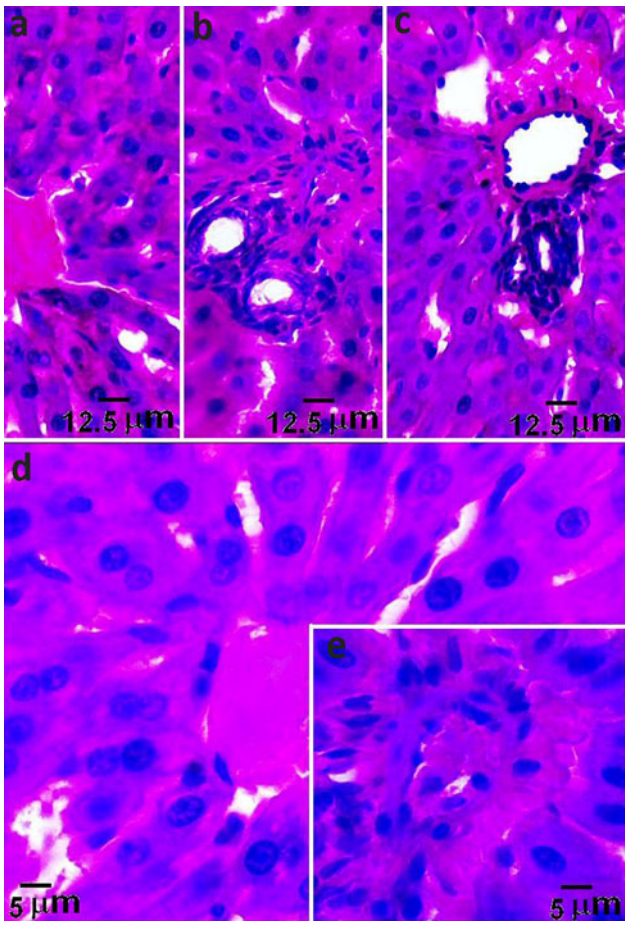

Fig. 7 Light microscopic photomicrograph of ovariectomy plus diabetic groups (Stain: Hemotoxylen Eosin). Sections were seen at different magnification and different areas. **a**, **d** central vein obstruction, **b–e** portal area

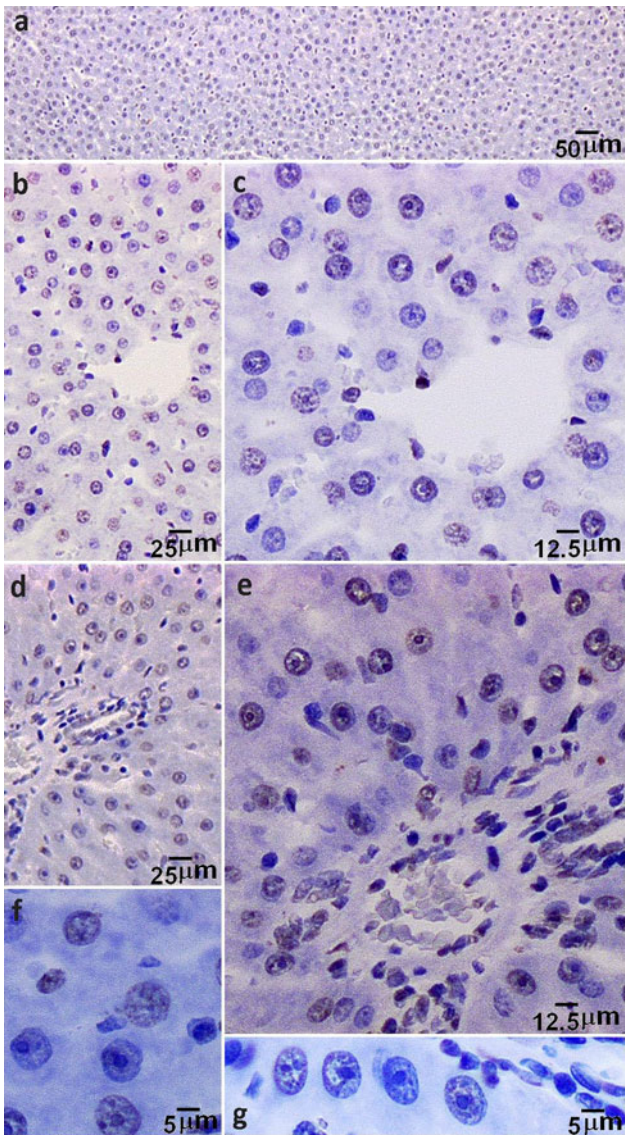

Fig. 8 Light microscopic photomicrograph of healty control groups (Stain: Tunel). Sections were showed at different magnification and different areas. **a–c** central vein, hepatocytes that radiate out from the central vein and sinusoids. **d–g** portal area, peri portal structures and hepatocytes

There were two remarkable findings in the diabetic group: the presence of apoptotic cells in the lobule and the increase in the numerical density of hepatocytes. The findings of increased hepatocytes and increased apoptosis conflict with one another, so we needed to determine the physiopathological mechanisms responsible for the increase in the number of hepatocytes and apoptosis.

We investigated tissue biochemistry parameters that directly indicated cellular damage in the liver. In this context, a marked increase was determined in the levels of both LPO and MPx, which were strong indicators of oxidative stress (at the beginning stage) and cell membrane injury (in the advanced period) in all experimental groups. When liver injury was detected, a response was observed in enzymatic antioxidants, such as SOD and CAT, and non-enzymatic antioxidants, such as GSH. Indeed, compared with control, we found a linear increase inCAT activity in the diabetic, OVX and OVX plus diabetes groups. In addition, we found a decrease in SOD activity and the amount of GSH in the diabetic group and an increase in the OVX group compared with controls.

When we evaluated all biochemical parameters together (i.e., SOD, GSH, CAT, LPO and MPx), we came to the conclusion that these were similar to one another in terms of degree of influence. Many studies have shown that OVX causes an increased oxidant capacity in the liver and brain tissues (Borras et al. 2003; Ozgonul et al. 2003). In addition, according to a study by Arteaga et al. 2003 estrogens have antioxidant properties and can inhibit lipid peroxidation in vitro. In the present study, LPO and MPx levels, which are indicators of oxidative stress in liver tissues,

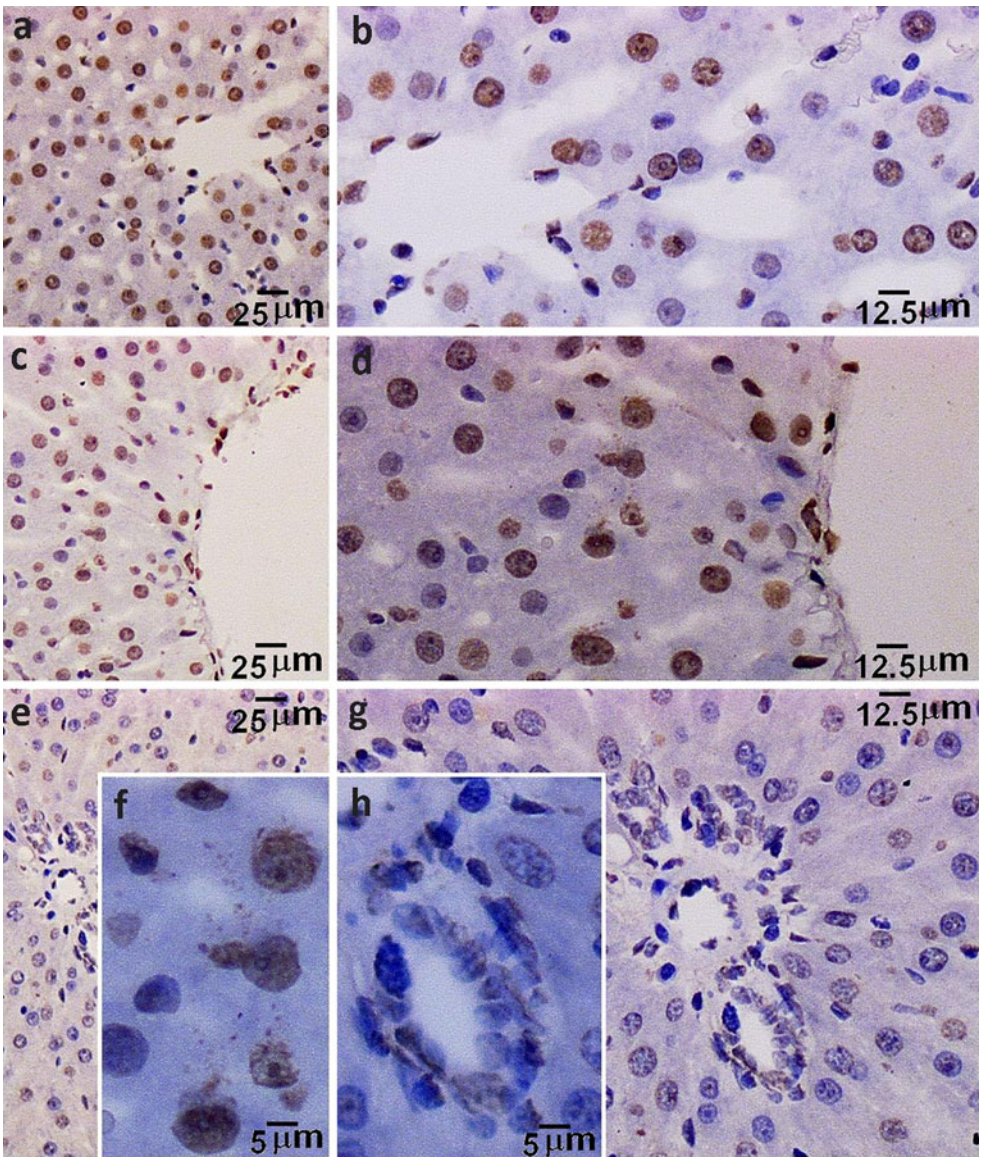

Fig. 9 Light microscopic photomicrograph of diabetic groups (Stain: Tunel). Sections were showed at different magnification and different areas. **a–d** tunnel positive, hepatocytes that radiate out from the central vein. **e–h** some tunnel positive hepatocytes that is located periportal area

were higher in the OVX group than in the control group. In addition, OVX rats presented an increase in antioxidant activities (i.e., SOD and CAT) in liver tissue. Furthermore, liver-reduced GSH may have been depleted due to the oxidative insult caused by OVX. Indeed, we found a decrease in sulfhydryl content in OVX rats, which suggested increased levels of oxidized protein and GSH. Increased activity of any enzyme could be linked to enhanced substrate production during the metabolic processes. Indeed, the increased CAT and MPx activities also suggested that the accumulation of hydrogen peroxide (H_2O_2) might be responsible for increased lipid peroxidation.

We also obtained interesting results in terms of SOD activity and GSH levels. The decreased SOD activity observed in the present study might indicate that diabetes results in an impaired ability to detoxify the superoxide radical via the SOD enzyme, which would cause an accumulation of the superoxide radical. Interestingly, diabetes caused a progressive decrease in GSH levels, whereas the OVX and OVX plus diabetes groups showed a significant increase compared with the control group ($P < 0.05$). These findings may have resulted because the adaptive ability of the body was insufficient to counter the toxic effects of diabetes. Organisms use GSH to eliminate H_2O_2 and other peroxides, and the increase in both CAT activity

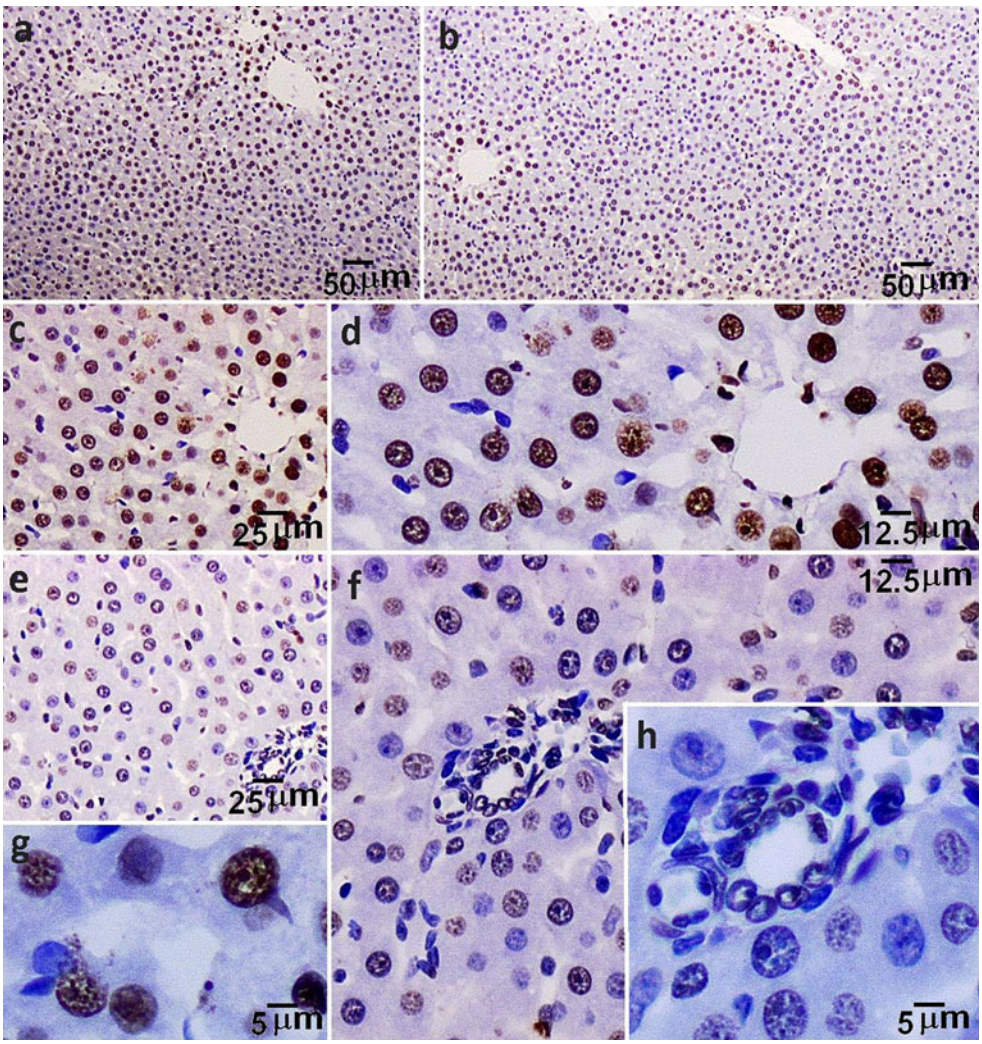

Fig. 10 Light microscopic photomicrograph of ovariectomy groups (Stain: Tunel). Sections were showed at different magnification and different areas. **a**–**d**, **g** tunnel positive hepatocytes that located central vein around. **e**, **f**, **h** some tunnel positive hepatocytes in periportal area

and GSH levels indicates the availability of a defense mechanism against ROS (Mayes and Botham 2003).

When we ignore observations and interpretations in the literature, the biochemical parameters, histopathological findings and quantitative estimations obtained in the present study suggest that the OVX- and diabetes-induced liver alterations are mediated by different mechanisms. The different mechanisms involved in OVX and diabetes, however, are unknown, and future studies are needed to determine these mechanisms and how they relate to each other. The present biochemical results suggest that an increase in cellular stress arising by different mechanisms in OVX plus diabetes leads to a decrease in the intracellular antioxidant defenses which were higher in OVX group. Another possibility is that a combination of mechanisms alters the production of oxidants, which causes cellular stress and consequent structural damage (Forgiarini et al. 2009).

Many experiments have shown that OVX reduces energy expenditure, which normally triggers increased adiposity and insulin resistance (Rogers et al. 2009; Saengsirisuwan et al. 2009). Estrogens do mediate physiological effects through two known estrogen receptors (ERs), alpha and beta (Hao et al. 2010; Simm et al. 2008). For example, 17β-estradiol (E2) binds ER_{alpha} with a higher affinity than ER_{beta} and promotes higher rates of ER_{alpha}-mediated transcriptional activity in the estrogen response elements (EREs). The liver is one of the well-established target tissues for estrogens (Hao et al. 2010). A previous study has indicated that estrogens influence glucose metabolism through the activation of ER_{alpha} (Riant et al. 2009). In a situation of generated oxidative stress,

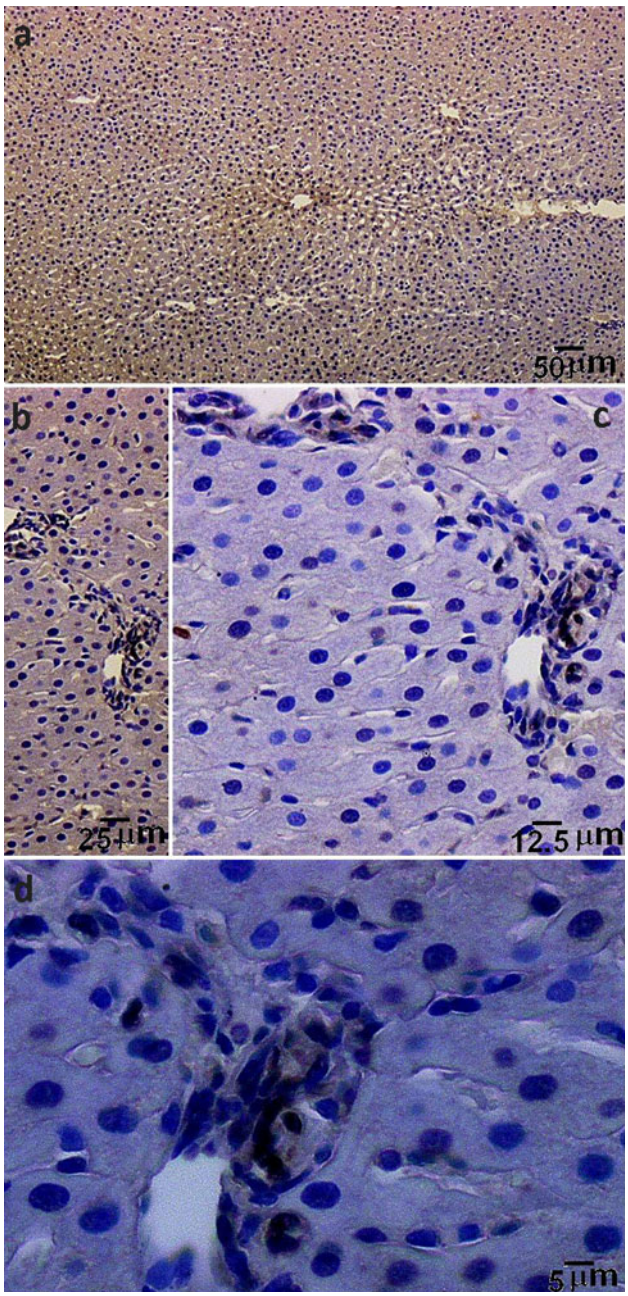

Fig. 11 Light microscopic photomicrograph of ovariectomy plus diabetic groups (Stain: Tunel). Sections were showed at different magnification and different areas. **a–d** some tunnel positive hepatocytes especially in bile duct

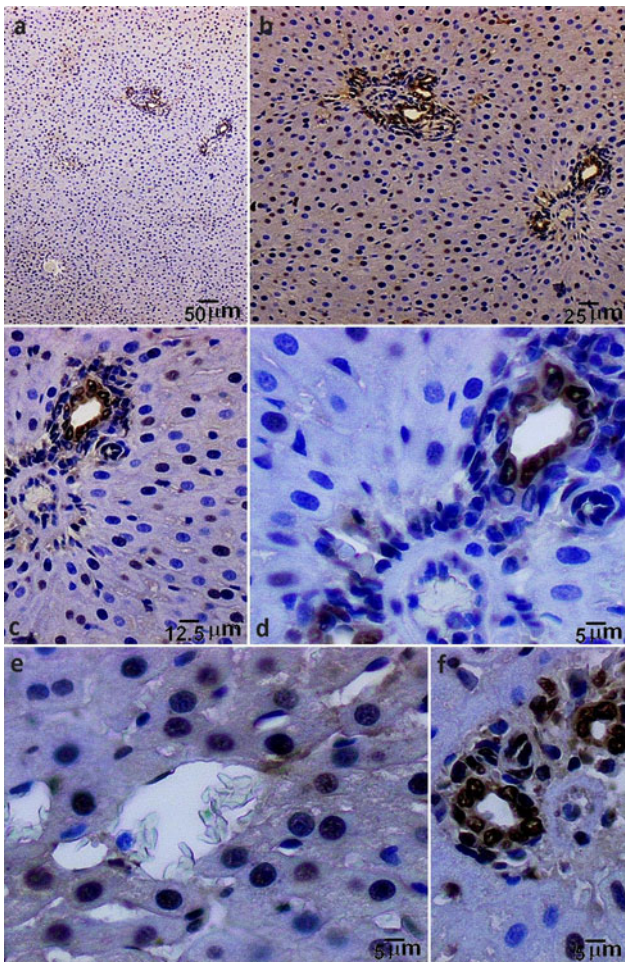

Fig. 12 Light microscopic photomicrograph of ovariectomy plus diabetic groups (Stain: Tunel). Sections were showed at different magnification and different areas. **a–d**, **f** some tunnel positive hepatocytes especially in bile duct in portal area. **e** tunnel positive hepatocytes around of central vein

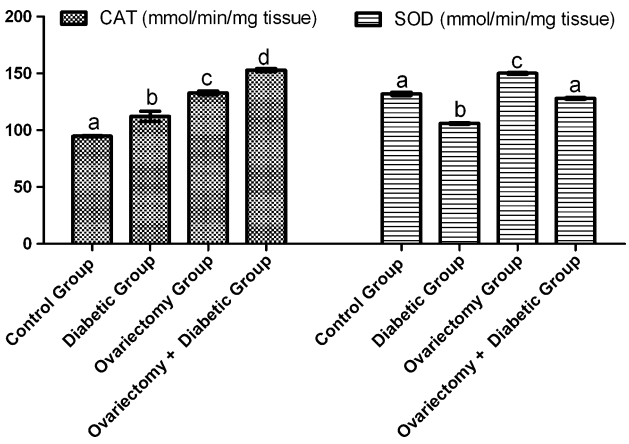

Fig. 13 CAT and SOD activities for all groups. Means in the same column by the same letter are not significantly different to the one way ANOVA Duncan test ($P < 0.05$). Results are expressed as mean ± standard deviation. (N: 6)

such as endurance training, ER transcript levels may appear to be able to adapt to new conditions in some situations via an ER-dependent mechanism (Paquette et al. 2007). It is clear that mitochondrial structures, including several messenger RNAs (mRNAs) for proteins of the respiratory chain encoded by both the nuclear and the mitochondrial genome (Solakidi et al. 2007), will be the first to be

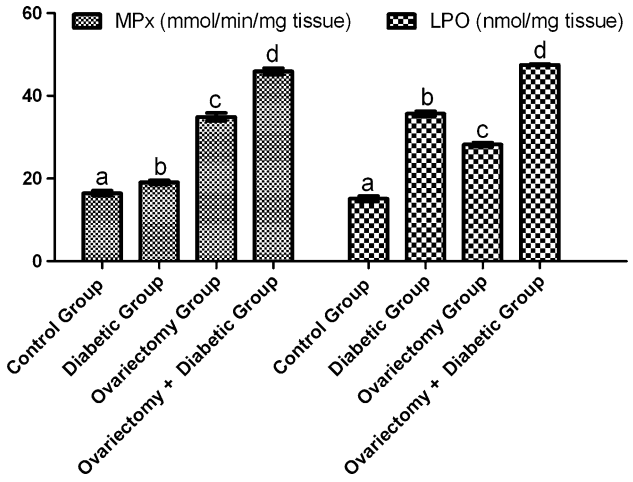

Fig. 14 Amount of LPO and activity of MPx results for all groups. Means in the same column by the same letter are not significantly different to the one way ANOVA Duncan test ($P < 0.05$). Results are expressed as mean ± standard deviation. (N: 6)

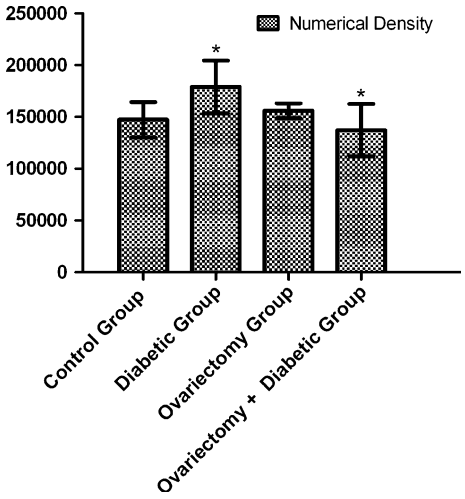

Fig. 16 Numerical density for all groups. Results are expressed as mean ± standard deviation in one way ANOVA LSD test. *$P < 0.05$ when compared to control group. (N:5)

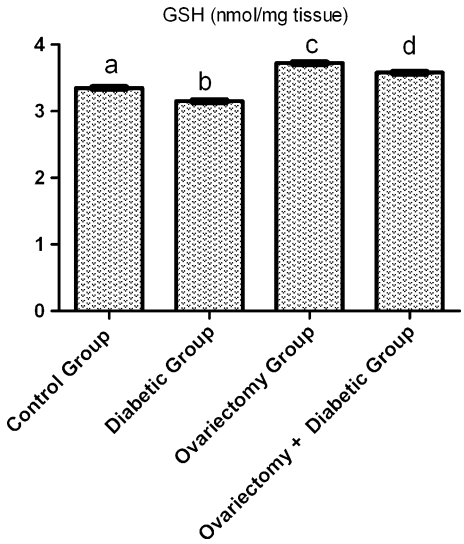

Fig. 15 Amount of GSH results for all groups. Means in the same column by the same letter are not significantly different to the one way ANOVA Duncan test ($P < 0.05$). Results are expressed as mean ± standard deviation. (N: 6)

affected by oxidative stress. Interestingly, in both OVX rats and ER_{alpha}-knockout (ERαKO) mice, mitochondrial structures were predominantly abnormal in shape, with abnormal cristae and loss of matrix area (Chen et al. 2005).

Serum lipid profiles are not only affected by diabetes but also by the production of membrane structures when it is time to renew old membranes and/or make new membranes. In cells, however, normal membrane structures do not protect normal features. Because insulin receptors are transmembrane proteins of the plasma membrane, changes in membrane structure would change the properties of insulin receptors. Thus, there cannot be a true relationship between the receptor and insulin. In addition, insulin resistance can alter oxidative stress. Previous studies have shown that metabolism tries to increase the beta cell number to release more insulin. In addition, hepatocytes may try to increase the amount of plasma membrane by way of cell division, which would explain the increase in hepatocytes. In terms of the cause of the apoptosis phenomenon in the diabetic group, cell division is a critical event that includes a lot of very complex processes and depends on a series of different factors that must progress in a healthy way. If there is both an increase in oxidative stress and a decrease in glucose uptake, hepatocytes are forced to divide, which would likely be triggered by an apoptotic mechanism.

In conclusion, the present study revealed several important findings:

1. oxidative stress occurs in the liver tissue at the end of postmenopausal aging and diabetes.
2. This stress causes necrosis in the liver,
3. triggers the apoptotic mechanism and
4. causes vessel damage, which may be linked to decreased amounts of estrogen as a result of menopausal aging.

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