Can vitamin E protect the pancreas against ethanol-induced alterations in adult male albino rats? A histomorphometric and immunohistochemical study

Ayman A. El-Enein Rizk[a], Shereen Abdel Fattah[a], Mohamed El Sebaie[b]

Departments of [a]Anatomy and Embryology, [b]Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Ayman Abo El-Enein Rizk, MD, Department of Anatomy and Embryology, Cairo University, Cairo, 12552, Egypt;
Tel: 020 01005157798;
e-mail: ayman.aboelenien@kasralainy.edu.eg

Received 24 January 2017
Accepted 26 February 2017

Kasr Al Ainy Medical Journal
2017, 23:43–53

Background
Ethanol has a destructive effect on the pancreas by inducing oxidative damage on pancreatic cells. This injurious effect could be ameliorated by the administration of vitamin E.
Aim
The aim of the study was to investigate the possible protective role of vitamin E on ethanol-induced alterations in the pancreas in adult male albino rats and also the effect of ethanol withdrawal.
Materials and methods
Forty adult male albino rats were divided into four equal groups (10 rats each): group I (control group), group II (ethanol-treated group), group III (ethanol and vitamin E-treated group), and group IV (recovery group). At the end of the experiment, the rats were sacrificed by cervical dislocation and their pancreas were excised and prepared for light microscopic and histochemical study. Detection of interleukin (IL)-1B and IL-6 by real-time PCR and proliferating cell nuclear antigen immunoexpression in the pancreatic tissue was carried out.
Results
Group II showed loss of acinar tissue, distorted degenerated pancreatic acini, dilated distorted interlobular ducts, distorted islets of Langerhans, and dilatation and congestion of the blood vessels. Interlobular inflammatory cellular infiltration was also detected. There was an increased collagen fiber deposition and diminution of the proliferating stem cells. There was a marked increase in IL-1B and IL-6 levels. Group III showed an obvious improvement in the structure of the pancreas. The recovery group showed improvement in the structure of the pancreas and minimal collagen deposition.
Conclusion
Vitamin E could protect the pancreas against ethanol-induced alteration in adult male albino rats.

Keywords:
ethanol, interleukin, oxidative stress, pancreas, vitamin E

Kasr Al Ainy Med J 23:43–53

1687-4625

Introduction

Chronic alcohol abuse is a major cause of pancreatitis; yet the mechanisms whereby ethanol exerts its toxic effects are not still understood. Prolonged alcohol intake at high doses causes cellular damage. Several researches have elucidated the destructive effects of ethanol on the pancreas and also that excessive alcohol intake is a major contributing factor of pancreatitis. Chronic pancreatitis is a disease characterized by irreversible damage to the pancreatic tissue in the form of fibrosis with progressive loss of both exocrine and endocrine architectures [1].

The acinar cells produce large amounts of digestive enzymes that in turn cause tissue damage. Ethanol is metabolized by the pancreatic acinar cells and the molecular alterations in these cells could lead to significant cellular injury [2].

Ethanol causes dose-dependent injury to the pancreatic tissue, characterized by edema, acinar vacuolization, and activation of trypsinogen. Whereas chronic infusion of ethanol causes marked pancreatic edema, inflammatory cellular infiltration, acinar necrosis, and fibrosis [3].

Pancreatic alcohol-induced fibrosis was assumed to be an active inflammatory process due to the expression of transforming growth factor-β1 and fibrosis-related cytokines produced by the pancreatic cells, accompanied by dynamic signals and cell-to-cell interactions. Pancreatic fibrosis also affects the pancreatic ducts, resulting in their

 DOI: 10.4103/kamj.kamj_6_17

dilatation and formation of pancreatic stones. Obstruction of the main pancreatic duct might lead to acute exacerbation of chronic pancreatitis, which could induce necrosis fibrosis sequence [4].

Fibrosis results in stricture of the main pancreatic duct, which, in turn, increases the pressure in the pancreatic duct, leading to abdominal pain. In addition to mechanical obstruction, pancreatic fibrosis also causes abdominal pain by affecting the nerves in the pancreas [5]. In addition to the exocrine (acinar cells) pancreas, endocrine pancreas (islet cells) is also affected by fibrosis. Despite this, diabetes only becomes evident at the later stages of the disease [6].

Alcohol-induced cytotoxicity could be attenuated by antioxidants that could suppress the apoptotic signals induced by other stimuli such as hypoxia, tumor necrosis factor-α (TNF-α), and oxidative stresses including nitric oxide, hydrogen peroxide, and superoxide [7]. Ethanol could induce apoptosis and inhibit pancreatic proliferation through oxidative stress and caspase formation [8]. On the other hand, continued alcohol administration increases pancreatic damage and cell necrosis. Meanwhile, alcohol withdrawal might prevent the progression of pancreatic lesion and apoptosis [9].

Ethanol administration augments nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in the pancreatic cells producing reactive oxygen species, explaining the role of ethanol-induced oxidative damage in the pancreatic cells. These injurious effects could be ameliorated by antioxidant administration assuring the role of antioxidants including vitamin E and *N*-acetyl-cysteine in inhibiting ethanol-induced injury [10]. Vitamin E plays a leading role in controlling excessive oxidative radical formation in cellular membranes including mitochondrial membranes [11].

The aim of the present study was to investigate the possible protective role of vitamin E on ethanol-induced alterations in the pancreas in adult male albino rats and also the effect of alcohol withdrawal.

Materials and methods

Animals

The present study was carried out on 40 Sprague-Dawley adult male albino rats weighing 200–220 g. They were obtained from the animal house, Faculty of Medicine, Cairo University. The rats were kept in separate cages (five/cage) and were maintained under standard laboratory conditions; diet and water were given *ad libitum*. The animals were acclimatized in the laboratory for 2 weeks before carrying out the experiment. All the animals were treated in accordance to the international guidelines for the care and use of laboratory animals. All procedures performed in the study were in accordance with the ethical standards of the institution of practice at which the studies were conducted.

Chemicals

(1) Ethanol (ethanol 50%): It was obtained from the Department of Biochemistry in the form of a glass bottle containing 1 l of 50% concentration of ethanol dissolved in saline (each 100 ml saline contains 50 g of ethanol). Each rat received ethanol (16 g/kg/day) orally through gastric gavage [12].
(2) Vitamin E: It was supplied in the form of 400 mg containing capsules obtained from the El Kahira Pharmaceutical Company. Vitamin E capsules were dissolved in olive oil to obtain a concentration of 4000 mg/100 ml (each 1 ml containing 40 mg of vitamin E). Each rat was administered vitamin E (600 mg/kg/day) orally through gastric gavage [13].

Experimental design

The rats were divided in to four equal groups (10 rats each):

(1) Group I (control group).
(2) Group II (ethanol-treated group), in which each rat received an oral dose of 50% ethanol dissolved in saline (16 g/kg/day) [12] orally through gastric gavage daily for 4 weeks.
(3) Group III (ethanol and vitamin E-treated group), in which each rat received an oral dose of 50% ethanol dissolved in saline (16 g/kg/day) [12] and vitamin E (600 mg/kg/day) [13] dissolved in olive oil daily for 4 weeks.
(4) Group IV (recovery group), in which each rat received an oral dose of 50% ethanol dissolved in saline (16 g/kg/day) by gastric gavage daily for 4 weeks, and then the animals were left to survive for another 4 weeks.

At the end of each experimental period, the rats were sacrificed by cervical dislocation. The abdomen of each rat was incised and the pancreas was excised and exposed by a pair of scissors; thereafter, the pancreas was washed with saline, and then pinned on a wax platform.

Methods

Histological study

Pancreatic sections were fixed in 10% formal saline for 24 h, and then processed, after which paraffin blocks were prepared. Paraffin sections of 5 μm were prepared and stained with hematoxylin and eosin for routine histological examination, and with Masson's trichrome for the detection of collagen fiber deposition [14]. The sections were subjected to a light microscopic examination, carried out in the Histology Department, Faculty of Medicine, Cairo University.

Immunohistochemical study

After deparaffinization, and rehydration and blocking of peroxidase, antigen retrieval was performed and sections in the peroxidase substrate solution were incubated overnight with primary antibodies against proliferating cell nuclear antigen (PCNA). Sections were deparaffinized, incubated, cooled, and then treated with 3% hydrogen peroxide solution, and then endogenous peroxide was inhibited. The material had been blocked with 5% BSA in PBS buffer (138 mmol/l NaCl, 30 mmol/l Na_2HPO_4, 2 mmol/l KH_2PO_4, pH 7.3) for 10 min and incubated with primary PCNA antibody (PC10; Abcam, United Kingdom) diluted in PBS buffer with 1% BSA (1 : 100 proportion) for 1 h. Horseradish peroxidase conjugated antimouse secondary antibodies (PC10; Abcam) were used for detection. Incubation was carried out for 30 min, followed by detection of antigens was carried out with 3,3′-diaminobenzidine (Sigma-Aldrich Chemical Co., Ltd, Saint Louis, Missouri, USA) used as chromogen. After immunohistochemical reaction, the sections were stained with hematoxylin, were dehydrated, immersed in xylene, and mounted. Positive results were obtained as deeply stained brown nuclei in the affected cells [15].

Image analyzer analysis

Mean area% of PCNA immunoexpression in the pancreatic cells:

The data were obtained by using the Leica Qwin 500 image analyzer computer system (Leica Qwin, England). The image analyzer consisted of a colored video camera, colored monitor, and a disc of IMB personal computer connected to the microscope, and was controlled by the Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Using the measuring field menu, the area, area%, and standard measuring frame were calculated. Measurements were carried out using an objective lens. Ten readings were obtained for each specimen.

Mean area% of collagen fiber deposition in the pancreatic tissue in Masson's trichrome-stained sections in all experimental groups:

Using the measuring field menu, the area, area%, and standard measuring frame of a standard area were calculated. In each chosen field the pancreatic tissue was enclosed inside the standard measuring frame and then the connective tissue (CT) area was masked by a blue binary color to be measured. The total area of collagen deposition in each specimen was measured and their mean values for each group were obtained.

Antioxidant enzymes analysis in pancreatic homogenate

Measurement of reduced glutathione (GSH) assay: GSH was estimated using the assay kit (Calbiochem, US) according to the manufacturer's instructions. The pancreatic homogenates were deproteinized in 5% metaphosphoric acid, and then centrifuged; the GSH contents of the supernatants were measured by the rate of colorimetric change of 5, 5′-dithiobis (nitrobenzoic acid) at 412 nm in the presence of GSH reductase and NADPH.

Real-time polymerase chain reaction study

Detection of interleukin (IL)-1b and IL-6 gene expression by real-time PCR:

(1) Isolation of pancreatic cells.
(2) RNA extraction: total RNA was extracted from the cell pellet using SV Total RNA Isolation System Kit (catalog no. Z3100; Promega, Madison, Wisconsin, USA).
(3) Complementary DNA synthesis: the extracted RNA was reverse transcribed into optical density using AMV Reverse Transcriptase (catalog no. M5101; Promega).
(4) Quantitative real-time PCR: the gene-specific forward and reverse primer pair was normalized. Each primer (forward and reverse) concentration in the mixture was 5 pmol/μl.

Sequence of the primers used for real-time PCR

	Primer sequence
IL-6	Forward: 5′-GCCCTTCAGGAACAGCTATGA-3′
	Reverse: 5′-CATCAGTCCAAGAAGGCAACT-3′
IL-1B	Forward: 5′-CAGGAAGGCAGTGTCACTCA-3′
	Reverse: 5′-AAAGAAGGTGCTTGGGTCCT-3′

The experiment and the following PCR program were set up:

(a) 50°C 2 min, 1 cycle.
(b) 95°C 10 min, 1 cycle.
(c) 95°C 15 s.
(d) 60°C, 30 s.
(e) 72°C 30 s, for 40 cycles.
(f) 72°C 10 min, 1 cycle.

A real-time PCR reaction mixture was 50 µl.

The following mixture was prepared in each optical tube:
(a) 25 µl SYBR Green Mix (2×).
(b) 0.5 µl cDNA.
(c) 2 µl primer pair mix (5 pmol/µl each primer).
(d) 22.5 µl H_2O.

(5) Data analysis: at the end of a quantitative real-time PCR running with SYBR Green chemistry, the relative quantification was assessed using the Applied Biosystem software (Applied Biosystem, US).

Statistical analysis

Data were coded and entered using the statistical package SPSS, version 23 (US). Data were summarized using mean and SD for quantitative variables. Comparisons between the experimental groups were carried out using analysis of variance [16]. *P*-values of less than or equal to 0.05 were considered as statistically significant and less than or equal to 0.001 was considered high significant. All the resultant data were tabulated and presented in graphs.

Results

Histological results

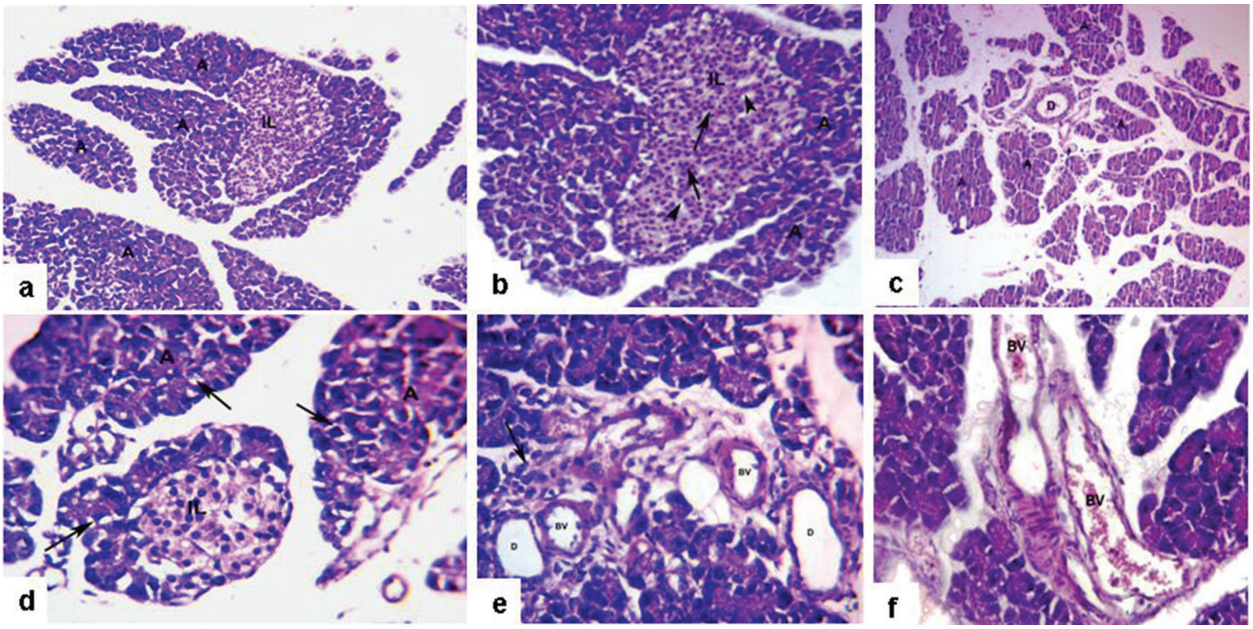
Group I (control group)

The pancreatic sections of group I revealed normal pancreatic acini and lightly stained islets of Langerhans (Fig. 1a). The pancreatic acini were formed of deeply stained acinar cells. The islets of Langerhans were formed as lightly stained small cells with pale cytoplasm (arrows) (Fig. 1b), interlobular duct lined by columnar cells (Fig. 1c). The rat pancreas revealed minimal collagen fiber deposition (Fig. 2a) and exhibited strong positive immunoexpression of PCNA reaction (Fig. 3a).

Group II (ethanol-treated group)

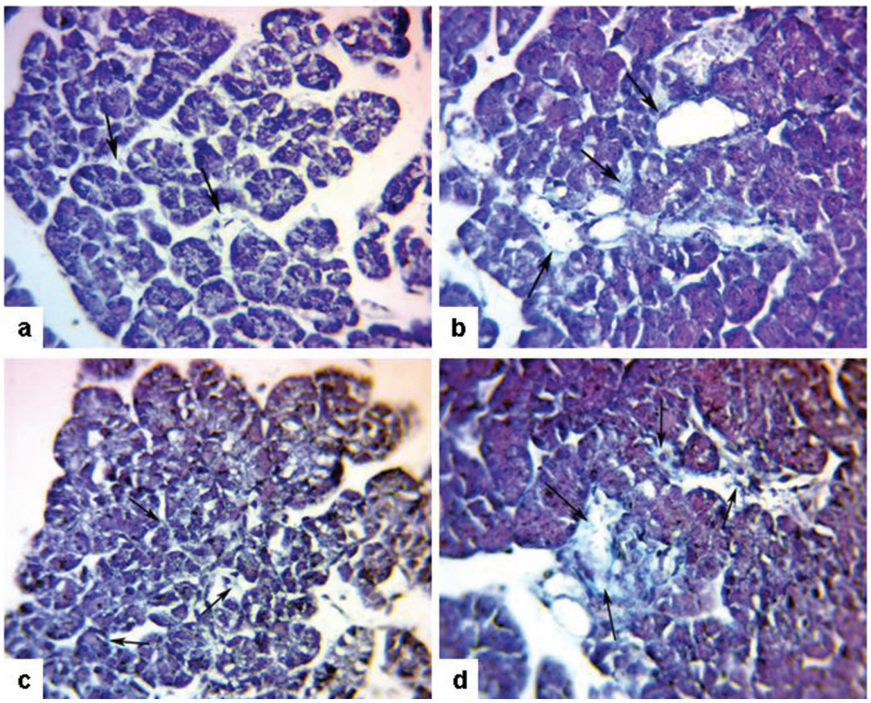
Group II showed an obvious damage of the pancreatic acinar architecture: distorted degenerated pancreatic acini, vacuolation of the acinar cells (arrows), distorted islets of Langerhans (Fig. 1d), dilated distorted interlobular ducts, interlobular inflammatory infiltration (Fig. 1e), and dilated congested blood vessels (Fig. 1f). The rat pancreas revealed increased fibrous tissue deposition (Fig. 2b) and exhibited mild positive immunoexpression PCNA reaction (Fig. 3b).

Figure 1



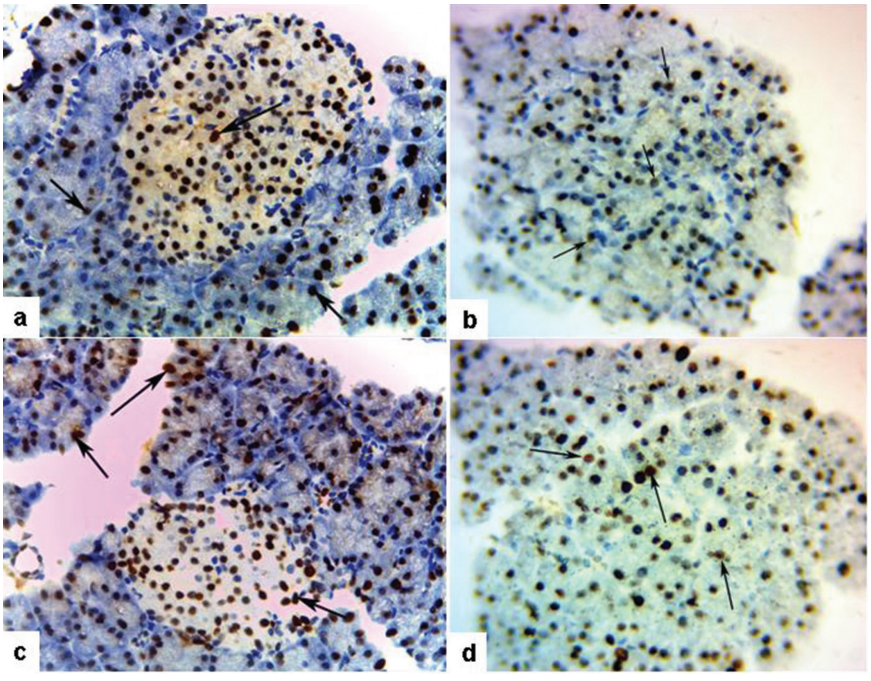
Photomicrographs of sections in rat pancreas of Group I (a) normal pancreatic acini (A) and islets of Langerhans (IL) (H&E ×100) (b) pancreatic acini (A) are formed of deeply stained acinar cells. Islets of Langerhans (IL) are formed of small cells with pale cytoplasm (arrows) (H&Ex400) (c) interlobular duct(D) (H&E ×400) Group II (d) distorted pancreatic acini (A) vacuolation of acinar cells (arrows) distorted islets of Langerhans (IL) (H&E ×400) (e) dilated interlobular ducts (D) interlobular inflammatory infiltration (arrow) (H&E ×400) (f) dilated congested blood vessels (BV) (H&E ×400)

Figure 2



Photomicrographs of sections in rat pancreas of Group I showing: (a) normal pancreatic acini (A) and islets of Langerhans (IL) (H&E ×100) (b) pancreatic acini (A) are formed of deeply stained acinar cells. Islets of Langerhans (IL) are formed of small cells with pale cytoplasm (arrows) (H&E ×400) (c) interlobular duct(D) (H&E ×400) Group II (d) distorted pancreatic acini (A) vacuolation of acinar cells (arrows) distorted islets of Langerhans (IL) (H&E ×400) (e) dilated interlobular ducts (D) interlobular inflammatory infiltration (arrow) (H&E ×400) (f) dilated congested blood vessels (BV) (H&E ×400).

Figure 3



Photomicrographs of sections of the rat pancreas of group I (a) showing minimal collagen fibers deposition (arrows). Group II (b) showing increased fibrous tissue deposition (arrows). Group III (c) showing minimal collage fiber deposition arrows. Group IV (d) showing moderate increase in collagen fibers deposition (arrows) (Masson'trichrome × 400).

Group III (vitamin E and ethanol-treated group)

Group III revealed preserved normal pancreatic architecture, pancreatic acini interlobular ducts (Fig. 4a), and islet cells of Langerhans (Fig. 4b). The pancreatic sections demonstrated minimal collagen fiber deposition arrows (Fig. 2c) and exhibited strong positive immunoexpression PCNA reaction (Fig. 3c).

Group IV (recovery group)

Sections of the rat pancreas of group IV showed normal pancreatic acini, mild congested dilated blood vessels (Fig. 4c), normally appeared islet cells of Langerhans, and dilatation of the interlobular ducts (Fig. 4d). The pancreatic sections revealed moderate increase in collagen fiber deposition (Fig. 2d) and exhibited strong positive immunoexpression PCNA reaction (Fig. 3d).

Histomorphometric analysis

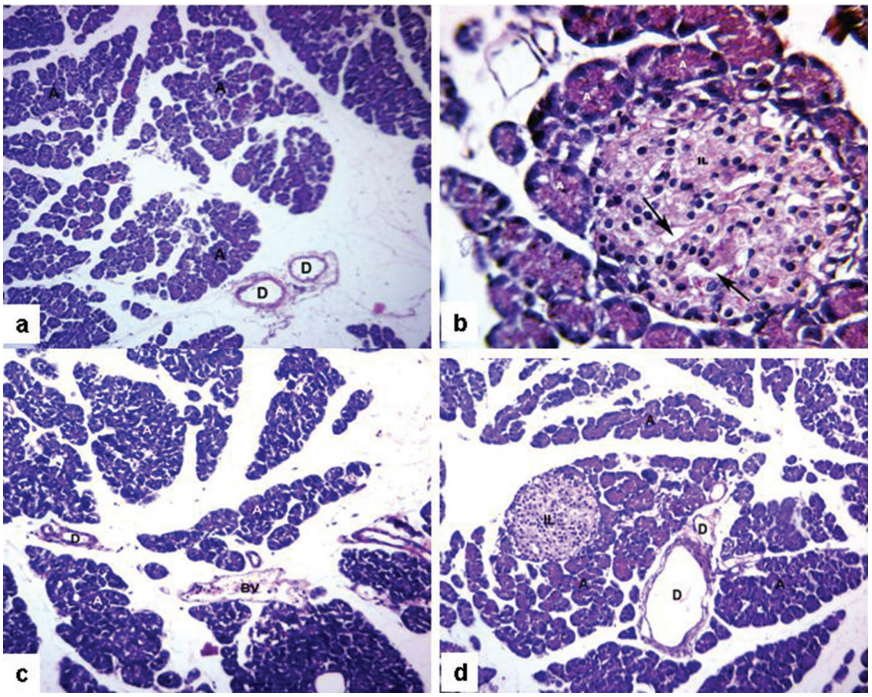
Statistical study of the mean area% of proliferating cell nuclear antigen

Histomorphometric analysis of the mean area percent of PCNA immune-expression in the pancreatic tissue in group II revealed a marked decrease in the mean area percentage of PCNA immune-expression (13.11± 1.50%), which was statistically highly significant

Table 1 Mean area% proliferating cell nuclear antigen immunoreactivity of the pancreatic tissue

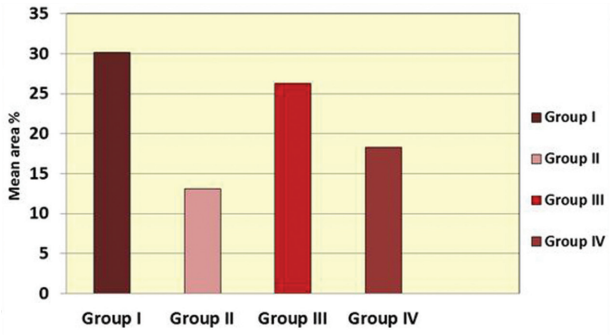
	Group I	Group II	Group III	Group IV
Mean±SD (%)	30.14±2.67	13.11±1.5	26.27±1.14	18.31±2.004
t-Value	0.614	4.966	1.452	3.144
P-value	–	0.0003**	0.1825	0.0137*

Figure 4



Photomicrographs of sections of the pancreatic tissue of Group I (a) exhibiting strong positive immunoexpression of PCNA reaction (arrows). Group II (b) exhibiting mild positive immunoexpression PCNA reaction (arrows). Group III (c) exhibiting strong positive immunoexpression PCNA reaction (arrows). Group IV(d) exhibiting strong positive immunoexpression PCNA reaction (arrows) (×400).

Figure 5



Showing the mean area % of PCNA immunoreactivity of the pancreatic tissue of all the experimental groups.

compared with the corresponding value in group I (30.14±2.67%). However, group III revealed an increase in the mean area% of PCNA immune-expression (26.27±1.114%), which was statistically insignificant compared with the same value in group I, and highly statistically significant as compared with group II. On the other hand, group IV showed reduction in the mean area% (18.13±2.004%), which was statistically significant as compared with the values of group I and group IV (Table 1 and Fig. 5).

The mean% of collagen fiber deposition in the pancreatic tissue

Histomorphometric analysis showed marked increase in the mean area of collagen deposition in the pancreatic tissue in group II (27.84±2.53%), which was statistically highly significant compared with the mean value of group I (8.62±0.36%). Meanwhile, group III revealed reversal of the value to nearly normal value (9.15±0.21%), which was statistically insignificant compared with group I. On the other hand, group IV showed increase in the mean area% of collagen fibers (12.42±3.72%), which was statistically significant as compared with the control group (8.62±0.36%) (Table 2 and Fig. 6).

Histochemical analysis (glutathione reductase enzyme)

The pancreatic homogenate showed that the mean value of GSH content in the pancreatic homogenate of group II was markedly decreased (0.06±0.02 nmol/mg protein), which was statistically highly significant as compared with the corresponding value of group I (0.34±0.14 nmol/mg protein). However, group III showed marked increase in GSH content (0.72±0.34 nmol/mg protein), which was statistically highly significant as compared with group I. Meanwhile, group IV showed reduction of GSH concentration (0.23±0.04 nmol/mg protein), which was statistically significant as compared with the control group (Table 3 and Fig. 7).

Estimation of interleukin-1B and interleukin-6 mean values (real-time polymerase chain reaction study)

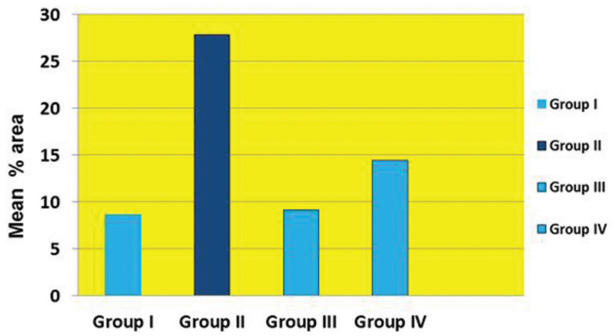
Mean value of interleukin-1B

The mean value of IL-1B of group II was markedly increased (92.80±8.29 pg/ml), which was statistically highly significant compared with group I. Meanwhile, vitamin E in group III showed reduction of this parameter to 37.90±8.74 pg/ml, which was statistically insignificant compared with the value in group I. Moreover, group IV showed marked increase in the mean value of IL-1B (60.12±9.77 pg/ml), which was statistically highly significant compared with group I.

Mean value of interleukin-6

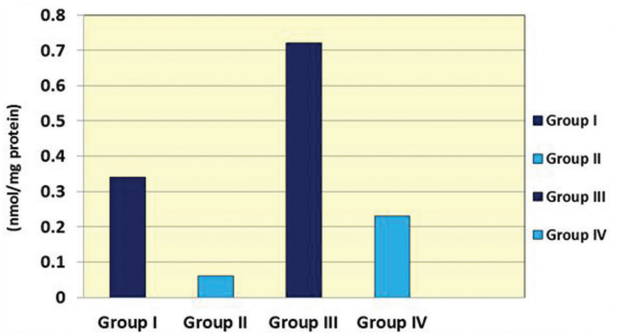
The mean value of IL-6 of group II was 75.12±12.08 pg/ml, which was statistically highly significant compared with group I (16.42±4.17 pg/ml). Meanwhile,

Figure 6



Showing the mean area % of collagen fibers deposition in the pancreatic tissue of all the experimental groups.

Figure 7



Showing the mean value of reduced glutathione (GTH) in the pancreatic tissue homogenate of all the experimental groups.

Table 2 Mean area% of collagen fibers deposition of all experimental groups

	Group I	Group II	Group III	Group IV
Mean±SD (%)	8.62±0.36	27.84±2.53	9.15±0.21	14.42±3.72
P-value	–	0.0005**	0.246	0.014*

Table 3 Mean value of glutathione concentration in of the pancreatic tissue

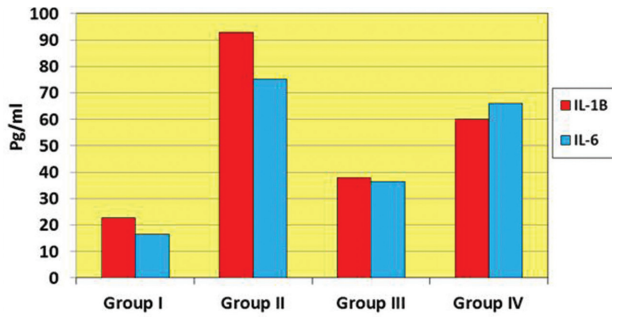
	Group I	Group II	Group III	Group IV
Mean±SD (nmol/mg)	0.34±0.14	0.06±0.02	0.72±0.34	0.23±0.04
P-value	–	0.0004**	0.0002**	0.024*

Table 4 Mean values of interleukin-1B and interleukin-6 of all experimental groups

	Group I	Group II	Group III	Group IV
IL-1B (mean±SD) (pg/ml)	22.82±6.04	92.80±8.29	37.90±8.74	60.12±9.77
P-value		0.00042**	0.016*	0.00062**
IL-6 (mean±SD) (pg/ml)	16.42±4.17	75.12±12.08	36.42±12.73	66.10±8.19
P-value		0.0006**	0.015*	0.0028**

IL, interleukin. *$P \leq 0.05$, statistically significant with control group. **$P \leq 0.001$, statistically highly significant with control group.

Figure 8



Showing the mean value of IL-1B and IL-6 in the pancreatic tissue of all the experimental groups.

group III revealed marked reduction of this parameter (36.40±12.73 pg/ml), which was statistically significant as compared with group I (16.42± 4.17 pg/ml). On the other hand, group IV showed marked increase in IL-6 value (66.10±8.19 pg/ml), which was statistically significant as compared with group I (16.42±4.17 pg/ml) (Table 4 and Fig. 8).

Discussion

Ethanol-induced cellular damage has been well studied in a variety of tissues including the liver, stomach, kidney, and small intestine. However, ethanol toxicity upon the pancreas was still debatable compared to other organs, although it is one of the principal target organs.

In the present study, group II showed a marked damage and distortion of the pancreatic acini. These findings were in agreement with Lee *et al.* [6] who related this acinar damage to the oxidative stress induced by ethanol. They added that ethanol is metabolized by three different pathways: alcohol dehydrogenase, microsomal ethanol oxidation system, and catalase. Each one of these pathways generates free radicals resulting in an imbalance leading to oxidative stress, which, in turn, could lead to peroxidation of the lipid bilayer of the cell membrane, which consecutively disintegrates the membrane.

On the other hand Zakhari [17], Wu *et al.* [18], and Shalbueva *et al.* [19], attributed the harmful effects of ethanol on the pancreatic acini to a nonoxidative pathway in which ethanol is converted to fatty acid ethyl ester, which has a critical effect on the acinar cells.

Moreover Lerch *et al.* [20], attributed this alcoholic damage of the acinar cells to the activation of pancreatic enzymes within the acinar cells, which in turn will be released in the interstitium, and resulted in the autodigestion of the pancreatic tissue. Poxleitner *et al.* [21] added that the release of these activated pancreatic enzymes into the circulation resulted in the development of multiple organ dysfunctions occurred.

Group II revealed dilatation of the interlobular ducts, which might be explained by Unal *et al.* [22] as a result of the toxic effect of ethanol metabolites, which in turn induces the release of digestive and lysosomal enzymes from the pancreatic acinar cells leading to pancreatic duct obstruction, and limitations of pancreatic regeneration and consequently dilatation of the ducts. These finding might be explained by Rotoli *et al.* [23] who reported that ethanol caused increase in the permeability of the main pancreatic ductal epithelium, and paracellular permeability of the pancreatic ductal cell line leading to ductal dilatation.

On the other hand, Braganza *et al.* [4] related the ductal dilatation to pancreatic fibrosis of the pancreatic ducts resulting in their dilatation and formation of pancreatic stones, due to inadequate drainage of pancreatic juice.

The present study revealed vacuolation of the cytoplasm of the pancreatic acinar cells in group II. The intracellular vacuoles could be attributed to either lipid accumulation or degranulated zymogen granules deposition within the cytoplasm of acinar and islet cells [3].

Moreover, group II showed dilatation and congestion of the pancreatic blood vessels, which could be explained by Kim *et al.* [24] who attributed that to the toxic effect of ethanol, which was

assumed to induce acute edematous pancreatitis, which might be converted into an acute necrotizing hemorrhagic resulting in obvious hemorrhage.

In the present study, group II revealed marked inflammatory cellular infiltration, evidenced by significant increase in the level of pancreatic IL-1B and IL-6. These findings were in agreement with Rossol *et al*. [25] and Huang *et al*. [26] who attributed this increase to the effect of ethanol, which increased the release of all inflammatory mediators and produced acinar cell-specific inflammatory responses and, consequently, the production of TNFα, IL-1β, IL-6, and IL-10, indicating that pancreatic acinar cells are the major source of proinflammatory and anti-inflammatory cytokines. Moreover, Gorelick [27] mentioned that ethanol could regulate transcription factors for inflammatory regulation such as nuclear factor κB and activator protein-1, inducing pancreatitis. Meanwhile, Dembele *et al*. [28] correlated the infiltration of inflammatory cells to the lymphocytes, which played the key role in the progression of pancreatic damage. Lymphocytes can be activated by gut-derived endotoxin and other stimuli to produce oxidants after alcohol consumption.

Group II revealed marked increase in the collagen deposition in the pancreatic tissue. This finding was in agreement with Yamamoto *et al*. [29] who reported that tissue fibrosis might result from an imbalance between its synthesis and degradation. The persistence of the alcoholic injury could lead to an increase in matrix production, resulting in a cumulative accumulation of extracellular matrix components. Moreover, Braganza *et al*.[4] related the mechanism of fibrous tissue formation to the destruction and damage of the acinar cells, which led to the deposition of type I collagen, which is considered as a characteristic feature of advanced chronic pancreatitis. Meanwhile, Alexander *et al*. [30] assumed that necrosis of the pancreatic tissue might result in cellular infiltration, and thus fibrosis could be a result of necrosis. This might be due to premature zymogen activation in the pancreatic acini as a result of recurrent attacks of acute alcoholic pancreatitis, which consequently leads to the development of chronic pancreatitis.

In the present study, group II demonstrated marked diminution of the proliferating stem cells and marked decrease in PCNA immunoreactivity in the pancreatic tissue, which could be explained by the results obtained by Hsu *et al*. [31] who attributed that to the effect of ethanol in suppressing cell growth factors and cell cycle proteins. Moreover, Ewa *et al*. [32] related the suppressed proliferative activity to the reduction in GSH levels, indicating the correlation between the oxidative stress and the suppression of PCNA. Meanwhile, Dembele *et al*. [28] attributed that to the toxic effect of ethanol, which inducted β-cell apoptosis and suppression of regeneration of the pancreatic cells, which might lead to mitochondrial dysfunction and oxidative stress.

Group II revealed marked reduction in the value of GSH in the pancreatic tissue, which was in agreement with the findings of Grattagliano *et al*. [7] who reported that ethanol caused distortion of the mitochondrial function and mitochondrial respiration, resulting in reduction in ATP synthesis and inhibition of mitochondria's ability to import GSH needed to protect against the oxidative damage. Thus, ethanol overall decreased the expression of cytosolic antioxidant enzymes including manganese superoxide dismutase, catalase, and GSH.

The pancreatic tissue of group III revealed an obvious improvement and preservation of the pancreatic acini, interlobular duct, and islet of Langerhans. These findings were in agreement with Masamune *et al*. [10] who reported that ethanol administration augments NADPH oxidase enzyme activity in the pancreatic cells producing reactive oxygen species, explaining the role of ethanol-induced oxidative damage in the pancreatic cells. Antioxidant administration could ameliorate such injurious effects and assuring the role of antioxidants intake including vitamin E and *N*-acetyl-cysteine in inhibiting ethanol-induced injury.

Improvement of the pancreatic structure was clear in group III, which was supported by Gomez *et al*. [13] who attributed that to the protective effect of vitamin E, which suppresses the oxidative stress induced by ethanol and thus protecting the pancreatic cells from free radicals production and enhancing cellular proliferation and regeneration. Moreover, Erejuwa [33] added that vitamin E was assumed to increase the level of the natural antioxidants produced by the body, which are needed in natural defensive mechanisms against free radicals and protection from oxidative stress. Carrasco *et al*. [34] added that antioxidant (including vitamin E) could

prevent the pancreas from oxidative process, lipid peroxidation, and protein oxidation, and enhance the production of antioxidant enzymes. Moreover, Jung *et al*. [35] and Gong *et al*. [36] assumed that antioxidants could inhibit the apoptotic pathways stimulated by other harmful stimuli such as hypoxia, TNF-α, and oxidative stresses including nitric oxide, hydrogen peroxide, and superoxide. Furthermore, Oruc *et al*. [37] confirmed the therapeutic role of antioxidants in the suppression of the inflammatory cytokines in animals with necrotizing pancreatitis.

In the present study, group III showed marked reduction of IL-1B and IL-6 levels in the pancreatic tissue. These findings were supported with Szabo *et al*. [38] and Jha *et al*. [39] who mentioned that antioxidants are beneficial in inhibition of nuclear factor κB activity as well as reduction of the proinflammatory cytokines such as TNF-α, IL-6, and IL-1, which might play the active role in ethanol-induced oxidative stress in the pancreas.

In the present study, group IV showed reversal of the changes in the pancreatic tissue and improvement of the histological picture of the pancreatic acini, interlobular ducts, and islets of Langerhans. There were minimal fibrous tissue deposition, increase in expression of the proliferating stem and GSH reductase in the pancreatic cells, and reduction of the inflammatory mediators (IL-1B and IL-6). These findings could be explained by Vonlaufen *et al*. [9] who attributed the recovery to the fact that alcohol withdrawal could minimize the cellular injury and cell death, and interrupt the fibrogenic process. On the other hand, Muller-Pillasch *et al*. [40] assumed that during the recovery period, there was an increase in the production of profibrogenic cytokines, matrix components, and also matrix metalloproteinases. They also reported that the final accumulation of collagen depends on the imbalance between its synthesis and degradation. They added that fibrous tissue formation depends on maintenance of the injury, which could lead to more cellular damage and excess collagen production and fibrosis. Therefore, cessation of the injury could inhibit the whole process and tissue damage [29].

It could be concluded that vitamin E could protect the pancreas against ethanol-induced alterations in adult male albino rats. The present study also assumed that this pancreatic injury could be reversible after cessation of ethanol administration.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Mas MR, Isik AT, Yamanel L, Inal V, Tasci I, Deveci S, *et al.* Antioxidant treatment with taurine ameliorates chronic pancreatitis in an experimental rat model. Pancreas 2006; 33:77–81.

2 Criddle DN, Raraty MG, Neoptolemos JP, *et al.* Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites. Proc Natl Acad Sci U S A 2004; 101:10738–10743.

3 Werner J, Saghir M, Warshaw AL, *et al.* Alcoholic pancreatitis in rats: injury from nonoxidative metabolites of ethanol. Am J Physiol Gastrointest Liver Physiol 2002; 283:65–73.

4 Braganza JM, Lee SH, McCloy RF, *et al.* Chronic pancreatitis. Lancet 2011; 377:1184–1197.

5 DiSebastiano P, DiMola FF, Buchler MW, *et al.* Pathogenesis of pain in chronic pancreatitis. Dig Dis 2004; 22:267–272.

6 Lee JH, Nguyen KH, Mishra S, *et al.* Prohibitin is expressed in pancreatic beta-cells and protects against oxidative and proapoptotic effects of ethanol. FEBS J 2010;277:488–500.

7 Grattagliano I, Palmieri V, Vendemiale G, *et al.* Chronic ethanol administration induces oxidative alterations and functional impairment of pancreatic mitochondria in the rat. Digestion 1999; 60:549–553.

8 Chavez PR, Lian F, Chung J, *et al.* Long-term ethanol consumption promotes hepatic tumorigenesis but impairs normal hepatocyte proliferation in rats. J Nutr 2011; 141:1049–1055.

9 Vonlaufen A, Phillips PA, Xu Z, *et al.* Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of LPS-induced pancreatic injury in alcohol-fed rats. Gut 2011; 60:238–246.

10 Masamune A, Watanabe T, Kikuta K, Satoh K, Shimosegawa T. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2008; 294:99–108.

11 Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signaling. J Nutr 2001; 131:369–373.

12 Takehiko U, Matthias F, Erwin G, Fuyumi I, Blair U, Bradford I, *et al.* Contribution of angiotensin II to alcohol-induced pancreatic fibrosis in rat. J Pharmacol Exp Ther Am Soc Pharmacol Exp Ther 2004; 311: 921–928.

13 Gomez JA, Molero X, Vaquero E, José-Antonio Gómez, Xavier Molero, Eva Vaquero, *et al.* Vitamin E attenuates biochemical and morphological features associated with the development of chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 2004; 287:162–169.

14 Bancroft J, Gamble M. Theory and practice 5th ed. Philadelphia: Churchill Livingstone; 2002.

15 Hall PA, Levison D, Woods A, Yu W, Kellock D, Watkins J, *et al.* Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol 1990; 162:285–294.

16 Chan YH. Biostatistics102: quantitative data – parametric & non-parametric tests. Singapore Med J 2003; 44:391–396.

17 Zakhari S. Overview: how is alcohol metabolized by the body? Alcohol Res Health 2006; 29:245–254.

18 Wu H, Bhopale K, Ansari G, Hai Wu, Kamlesh K, Bhopale GAS, *et al.* Ethanol-induced cytotoxicity in rat pancreatic acinar AR42J cells: role of fatty acid ethyl esters. Alcohol Alcohol 2008; 43:1–8.

19 Shalbueva N, Mareninova OA, Gerloff A, *et al.* Effects of oxidative alcohol metabolism on the mitochondrial permeability transition pore and necrosis in a mouse model of alcoholic pancreatitis. Gastroenterology 2013; 144:437–446.

20 Lerch MM, Albrecht E, Ruthenburger M, *et al.* Pathophysiology of alcohol induced pancreatitis. Pancreas 2003; 27:291–296.

21 Poxleitner PJ, Seifert G, Richter SC, *et al.* Infected pancreatic necrosis increases the severity of experimental necrotizing pancreatitis in mice. Pancreas 2013; 42:1150–1156.

22 Unal E, Uzun H, Kusaslan R, *et al.* A high-density lipoprotein-associated lipophilic antioxidant activity and lipid profile in experimental acute pancreatitis. Pancreas 2005; 31:84–87.

23 Rotoli B, Orlandini G, Guizzardi S, Uggeri J, Dall'Asta V, Gazzola G, *et al.* Ethanol increases the paracellular permeability of monolayers of CAPAN-1 pancreatic duct cells. J Mol Histol 2004; 35:355–362.

24 Kim HS, Christine C, Christopher C. Review of experimental animal models of acute pancreatitis. HPB (Oxford) 2006; 8:264–286.

25 Rossol M, Heine H, Meusch U, Quandt D, Klein C, Sweet M, *et al.* LPS-induced cytokine production in human monocytes and macrophages. Crit Rev Immunol 2011; 31:379–446.

26 Huang H, Liu Y, Daniluk J, Gaiser S, Chu J, Wang H, *et al.* Activation of nuclear factor-kappaB in acinar cells increases the severity of pancreatitis in mice. Gastroenterology 2012; 144:202–210.

27 Gorelick FS. Alcohol and zymogen activation in the pancreatic acinar cell. Pancreas 2003; 27:305–310.

28 Dembele K, Nguyen KH, Hernandez TA, Nyomba G. Effects ofethanol on pancreatic beta-cell death: interaction with glucose and fatty acids. Cell Biol Toxicol 2009; 25:141–152.

29 Yamamoto A, Ishiguro H, Ko SB, *et al.* Ethanol induces fluid hypersecretion from guinea-pig pancreatic duct cells. J Physiol 2003; 551:917–926.

30 Alexander A, Aghdassi JM, Sandra C, *et al.* Animal models for investigating chronic pancreatitis. Fibrogenesis Tissue Repair 2011; 4:26.

31 Hsu MK, Qiao L, Ho V, *et al.* Ethanol reduces p38 kinase activation and cyclin D1 protein expression after partial hepatectomy in rats. J Hepatol 2006; 44:375–382.

32 Ewa W, Anna D, Maksymilian K, Ewa F, Wojciech P, Monika S, *et al.* Exposure to ethanol and tobacco smoke in relation to level of PCNA antigen expression in pancreatic and hepatic rat cells. Pharmacol Rep 2013; 65:914–926.

33 Erejuwa O. Antioxidant protective effect of glibenclamide and metformin in combination with honey in the pancreas of streptozotocin-induced diabetic rats. Int J Mol Sci 2010; 11:2056–2066.

34 Carrasco C, Rodriguez AB, Pariente JA. Effects of melatonin on the oxidative damage and pancreatic antioxidant defenses in cerulein-induced acute pancreatitis in rats. Hepatobiliary Pancreat Dis Int 2014; 13:442–446.

35 Jung JY, Han CR, Jeong YJ, Kim H, Lim H, Lee K, *et al.* Epigallocatechin gallate inhibits nitric oxide-induced apoptosis in rat PC12 cells. Neurosci Lett 2007; 411: 222–227.

36 Gong G, Qin Y, Huang W, *et al.* Rutin inhibits hydrogen peroxide-induced apoptosis through regulating reactive oxygen species mediated mitochondrial dysfunction pathway in human umbilical vein endothelial cells. Eur J Pharmacol 2010; 628:27–35.

37 Oruc N, Ozutemiz AO, Yukselen V, *et al.* A new therapeutic agent in acute pancreatitis?. Pancreas 2004; 28:1–8.

38 Szabo G, Mandrekar P, Oak S, *et al.* Effect of ethanol on inflammatory responses. Implications for pancreatitis. Pancreatology 2007; 7:115–123.

39 Jha RK, Ma Q, Sha H, *et al.* Emerging role of resveratrol in the treatment of severe acute pancreatitis. Front Biosci 2010; 2:168–175.

40 Castaño G, García-Unzueta M, Domínguez-Diez A, Fernández-González M, García A, Mayorga-Fernández M, *et al.* Pancreatic fibrosis in rats and its response to antioxidant treatment. J Pancreas 2005; 6:316–324.