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ORIGINAL ARTICLE

Effect of Vitamin E on Ethanol Induced Exocrine Pancreatic Injury in Adult Male Albino Rat: Light, Electron Microscopic and Biochemical Study

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ABSTRACT

Background: Excessive alcohol intake is a leading factor of chronic pancreatitis. Chronic administration of ethanol causes marked pancreatic edema, inflammatory cellular infiltration, acinar necrosis, and fibrosis. Vitamin E is a fat-soluble vitamin that helps in the formation of red blood cells and the prevention of oxidation in the body. The current study aimed to compare the protective effect of vitamin E and withdrawal from ethanol in the ethanol-induced injury of the exocrine pancreas. **Methods**: Fifty adult male albino rats were divided into five equal groups: (control, sham control, ethanol-treated group, ethanol, and vitamin E-treated group, and withdrawal group). Light microscopic examination was done using hematoxylin and eosin and Masson's trichrome stains. Electron microscopic examination was carried out using transmission electron microscopy. Serum level of lipase and alpha-amylase was measured in blood samples. Measurements of malondialdehyde (MDA) and superoxide dismutase (SOD) activity in pancreatic tissue homogenates were performed.**Results:** Examination of specimens of ethanol-treated rats revealed markedly disturbed pancreatic architecture, pancreatic acini, widening of spaces, dilated interlobular ducts, pyknotic nuclei, destruction of mitochondria with loss of cristae, and extensive fibrosis. These morphological changes were associated with a significant increase in serum lipase, alpha-amylase, MDA and a decrease in SOD activity. Ethanol and vitamin E co-administration markedly ameliorated these histological alterations, but withdrawal from ethanol could not improve its harmful effects. **Conclusions:** This study revealed that concomitant administration of vitamin E with ethanol could significantly improve ethanol-induced exocrine pancreatic damage, possibly due to its antioxidant property.**Key words:** Vitamin E; Ethanol; Pancreas;Exocrine; Antioxidant

INTRODUCTION

Excessive alcohol intake is a leading factor in the pathogenesis of chronic pancreatitis. It is characterized by severe damage to the pancreatic tissue in the form of fibrosis with progressive loss of pancreatic architectures. Ethanol is metabolized by pancreatic acinar cells and molecular alterations in these cells could lead to marked cell damage. The pancreas can metabolize ethanol by means of oxidative and nonoxidative pathways to generate metabolites, such as acetaldehyde and fatty acid ethyl esters [1-3].

Chronic administration of ethanol causes marked pancreatic edema, inflammatory cellular infiltration, acinar necrosis, and fibrosis. Individuals suffering from chronic pancreatitis have a 20-fold greater likelihood of developing pancreatic cancer. Because one of the cardinal characteristics of chronic pancreatitis is aberrant tissue repair, which results in fibrotic scarring, ethanol consumption alters pancreatic repair. Moreover, ethanol may have an indirect role in the initiation of pancreatic cancer. Thus, the effects of ethanol on the repair of the damaged pancreas may be a contributing factor in pancreatic cancer and alcoholic pancreatitis. Chronic pancreatitis can result from recurrent attacks of acute pancreatitis resulting in permanent structural and functional damage and a high risk of development of pancreatic cancer [4-6].

Vitamin E is a fat-soluble vitamin that includes four tocopherols (α, β, δ, γ) and four tocotrienols (α, β, δ, γ). It helps in the formation of red blood cells and the prevention of oxidation in the body. It also assists in the protection of the lungs from becoming damaged

by air pollutants. This vitamin can be found in whole grains e.g., wheat and oat, wheat germ, green leafy vegetables, sardines, egg yolks, nuts, bread, cereals, and seeds. The deficiency of this vitamin can cause several diseases like cancer, diabetes, heart disease, and Alzheimer's disease. Both tocopherols and tocotrienols are important components of biological membranes having both antioxidant and nonantioxidant effects [7, 8]. Vitamin E is one of the main lipid-soluble antioxidant vitamins. It is present in membranes and lipoproteins, so it is regarded as a membrane antioxidant. It can stop the chain reaction of lipid peroxidation by scavenging intermediate peroxyl radicles. It might protect the liver and other organs against toxic materials like chemotherapy, ozone, and radiotherapy in a dose-dependent manner. Vitamin E has a role in the inhibition of oxidation of polyunsaturated fatty acids, neurological functions, and prevention of platelet aggregation [9]. The aim of the present work was to elucidate the potential role of vitamin E against ethanol-induced exocrine pancreatic damage, highlighting the antioxidant mechanism of vitamin E in this perspective and the effect of ethanol withdrawal.

METHODS

Chemicals: Ethanol (ethanol 50%) was obtained from the Department of Biochemistry, Faculty of Medicine, Cairo University, in the form of a glass bottle containing one Litre of 50% concentration of ethanol dissolved in saline (each 100 ml saline contains 50 g of ethanol) [10]. **Vitamin E** was supplied in the form of 400 mg containing capsules obtained from El Kahira Pharmaceutical Company, Cairo, Egypt. **Vitamin E** capsules were dissolved in olive oil to obtain a concentration of 4000 mg/100 ml (each 1ml containing 40 mg of vitamin E) [11].

Sample size calculation:

The sample size was calculated based on a pilot study of three rats in each group; the difference between cases and controls in serum superoxide dismutase (SOD) was 0.3±0.08. Nine rats were needed in each group to achieve power 80% and 5% significance level. Sample size calculation was achieved using OpenEpi, Version 3, open-source calculator.

Animals and experimental design:

Fifty adult male albino rats weighing 180-250 g were used in this study. They were obtained from the animal house, Faculty of Medicine, Cairo University. The experiment was carried out in agreement with the guidelines of the Cairo University of Medical Sciences for the care and use of laboratory animals. The rats were housed in separate cages rats (2-4 per cage) and maintained under standard laboratory and environmental conditions with standard rat chew. In the study, only male animals were used; females were excluded to avoid the possible influence of hormonal changes in the estrous cycle on the results achieved [12].

The rats were divided into five groups, 10 rats in each:

Group I (control group): The rats received 0.9% saline orally by oral gavage once daily for four weeks.

Group II (sham control group): They received olive oil (vehicle of vitamin E) orally by oral gavage once daily for four weeks.

Group III (ethanol-treated group): The animals received an oral dose of 50% ethanol dissolved in saline (16 g/kg/day) orally by oral gavage daily for four weeks [10].

Group IV (ethanol & vitamin E treated group): They received ethanol as in group III, and vitamin E (600 mg/kg/day) dissolved in olive oil daily for four weeks [11].

Group V (withdrawal group): The animals received ethanol as in group III, and then they were left to survive for another four weeks, with only food and water ad libitum.

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; thereafter, the pancreas was washed with saline and prepared for light, electron microscopic study and biochemical assessment.

The pancreas was excised and processed for the following studies:

1- Light microscopic study [13]:

A- **Hematoxylin and eosin stain (H&E):** The pancreatic tissues were fixed in 4% para-formaldehyde. The histological sections were deparaffinized, rehydrated, and washed in running water. The sections were then immersed in Harris' hematoxylin for two minutes, washed in running water (five minutes), rinsed in distilled water (one minute), stained in an aqueous solution of eosin (five minutes), and dehydrated in ascending concentrations of ethanol. The sections were cleared in xylene and mounted under a cover slip.

B- Masson's trichrome staining:

The sections were deparaffinized, rehydrated, washed in running water (two minutes), immersed in 5% iron alum (ten minutes) and Regaud's hematoxylin (three minutes), and rinsed in distilled water, 95% alcohol, and picric alcohol. Then, they were washed in running water (ten minutes),

immersed in xylidine ponceau aqueous solution (three minutes), distilled water and 1% glacial acetic acid, and 1% phosphomolybdic acid (three minutes). They were rinsed again in distilled water, immersed in aniline blue for 2-5 minutes, dehydrated, cleared, and mounted under a cover slip.

2- Electron microscopic study [14]:

Pieces from the pancreas were subjected to ultrastructural examination through the preparation of semithin sections and observed with an optic microscope to choose the regions of interest. The chosen regions were cut and put into square and eyelet grids. Examination of the processed sections was performed by JEOL JEM 1010 transmission electron microscope (TEM) in the Electron Microscope Research Laboratory, Faculty of Agriculture Research Center, Cairo University.

3- Biochemical assessment:

A- Serum lipase and alpha-amylase:

Blood samples were obtained from tail vein of the rats before scarification. Assessment of serum alpha-amylase and lipase were performed according to the manufacturer's instructions using reagent kits (Spectrum Diagnostics Company, Cairo, Egypt) [15, 16]. Alpha amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl1-galactopyranosyl maltoside (GALG2-CNP) to glucose polymers and p-nitrophenyl oligosaccharide at short chain producing 2-chloro4-nitrophenol (CNP). The increased extinction can be measured by spectrophotometry at 405 nm and results are proportional to the activity of alpha-amylasepresenting the sample. Regarding lipase determination, synthetic substrate (DGMRE) is split by lipase to yield the coloured final product methylresorufin. The increasing absorbance of the red methylresorufin is measured photo-metrically.

B- Malondialdehyde (MDA) and superoxide dismutase (SOD):

Malondialdehyde is a biomarker for lipid peroxidation. It was assessed in tissue homogenate according to Buege and Aust [17]. A 100 mg pieces of pancreatic tissue were homogenized in 1 mL of PBS, PH 7.0 with a micro pestle in a microtube. Trichloroacetic acid (20%) was added to tissue homogenate to precipitate the protein and centrifuged. Collection of the supernatants was carried out and the addition of thiobarbituric acid solution (0.8%) was done. A water bath was used for boiling for 10 minutes, then the absorbance was measured at 405 nm. Assessment of the concentration of MDA in the homogenate supernatant was done utilizing the standard curves.

The superoxide dismutase is an antioxidative stress marker. Assessment of SOD activity in tissue homogenate was performed according to Weydert and Cullen [18]. The reduction of nitroblue tetrazolium was arrested by O2, which is generated by the xanthine/xanthine oxidase system. One unit is the amount of SOD that inhibits the rate of formazan dye formation by 50%.

Statistical Analysis:

The collected data was statistically analyzed using SPSS software statistical computer package version 22 (SPSS Inc, USA). The mean value and standard deviation (SD) were calculated. One-way ANOVA (Analysis of variance) was performed to test the difference between groups as regards mean values of measured variables; Tukey (Post hoc test) was used for multiple comparisons between pairs of groups. For interpretation of results of tests of significance, significance was accepted at $P \leq 0.05$.

RESULTS

***Histological results*:**

Groups II (sham control):

Light and electron microscopic examination of pancreas specimens of control and sham control groups exhibited similar findings. Hematoxylin and eosin (H&E) stained sections showed normal pancreatic architecture; pancreatic exocrine acini arranged in lobules separated by narrow septa. Interlobular duct was observed and blood vessels were seen filled with blood cells **(Fig. 1 A)**.

Masson's trichrome-stained sections showed very fine connective tissue fibers in between the acini of the pancreas, around the interlobular duct, and around blood vessels **(Fig. 1 B)**. Electron microscopic examination revealed acinar cells with euchromatic nuclei, well-developed cisternae of rough endoplasmic reticulum, and numerous electron-dense secretory granules **(Fig. 1 C)**.

Group III (ethanol-treated group):

Light microscopic examination of H&E-stained sections showed loss of architecture of pancreatic acini, widening of spaces, dilated interlobular duct, inflammatory cell infiltration, and pyknotic nuclei **(Fig. 2A)**. Sections stained with Masson's trichrome showed excessive collagen fibers around pancreatic acini, ducts, and blood vessel **(Fig. 2B)**. Electron microscopic examination revealed acinar cells with indentation of nuclei, clumping of chromatin, cytoplasmic rarefaction. Destruction of mitochondria with loss of cristae, dilated rough endoplasmic reticulum, and decreased secretory granules **(Figs. 2C, 2D)**.

Group IV (ethanol & vitamin E treated group):

Light microscopic examination of pancreas specimens stained with H&E showed normal architecture of some acini and loss of architecture of other acini and there were wide spaces between them **(Fig. 3A)**. Masson's trichrome stained sections showed minimal collagen fibers around acini, ducts, and blood vessels **(Fig. 3B)**. Electron microscopic examination revealed acinar cells having euchromatic nuclei, abundant secretory granules, rough endoplasmic reticulum were apparently normal but few of them were dilated **(Fig. 3C)**.

Group V (withdrawal group):

Light microscopic examination of H&E-stained sections showed loss of architecture of pancreatic acini, widening of spaces, dilated interlobular duct, hypertrophied blood vessel, inflammatory cell infiltration, and pyknotic nuclei **(Fig. 4A)**. Sections stained with Masson's trichrome exhibited excessive collagen fibers around pancreatic acini, dilated interlobular ducts around a blood vessel **(Fig. 4B)**. Electron microscopic examination revealed acinar cells degenerated indented nuclei, blebbing of the nuclear envelope, clumping of chromatin, extensive cytoplasmic rarefaction, dilated rough endoplasmic reticulum extremely decreased secretory granules with the destruction of mitochondria with loss of cristae **(Figs. 4C, 4D)**.

Biochemical results:

Serum lipase:

Serum lipase had a statistically significantly higher level in group III (79.8 ± 11.1) and V (76.8 ± 11.6) when compared to the control group (29.8 ± 4.1) and sham control group (30.01 ± 4.13), p < 0.0001. However, the difference between group IV and both control and sham control groups was not statistically significant, p = 0.182, p = 0.191, respectively. There was a statistically significant decrease in group IV (41.2 ± 3.1) when compared to group III (79.8 ± 11.1) (p < 0.0001). There was a non-significant difference between the group V and group III (p = 0.941). Also, there was a statistically significant increase in group V (76.8 ± 11.6) when compared to group IV (41.2 ± 3.1) (p < 0.0001) **(Table 1, Figure 5A)**.

Alpha-amylase:

Alpha-amylase had a statistically significantly higher level in group III (106.2 ± 15.8) and V (104.4 ± 12.6) when compared to control (50.4 ± 8.5) and sham control (51.03 ± 8.59) groups, p < 0.0001. The difference between group IV and control as well as sham control groups was not statistically significant (p=0.308, p =0.316, respectively). There was a statistically significant decrease in group IV when compared to group III (63.2 ± 4.9 vs. 106.2 ± 15.8) (p < 0.0001). A non-significant difference was found between group III and group V (p = 0.994). Alpha-amylase was significantly higher in group V (104.4 ± 12.6) compared to group IV (63.2 ± 4.9) (p < 0.0001) **(Table 2, Figure 5B)**.

Malondialdehyde (MDA):

Malondialdehyde was statistically significantly higher in group III (58.95 ± 7.99) when compared to both control (9.93 ± 2.87) and sham control (9.91 ± 2.91) groups, p <0.0001. However, the difference between group IV and both control and sham control groups was not statistically significant (p=0.975, p =0.981, respectively). There was a statistically non-significant difference between group III and group V (p = 0.883). Moreover, MDA was significantly higher in group V (55.88± 11.6) compared to group IV (10.12± 2.25) (p = 0.002) **(Table 3, Figure 5C)**.

Superoxide dismutase (SOD):

Superoxide dismutase was statistically significantly higher in group IV (2.82± 0.42) when compared to group III (0.86 ± 0.26) (p < 0.0001). The difference between group IV and both control and sham control groups was non-statistically significant (p=0.697, p =0.701) respectively. There was a non-significant difference between group III and group V (p = 0.789). Superoxide dismutase was significantly lower in group V (0.92 ± .29) than in group IV (2.82 ± 0.42) (p = 0.004) **(Table 4, Figure 5D)**.

Table 1: pairwise comparisons of the mean serum lipase level obtained from different groups of the examined animals

Serum lipase	Group I (Control)		Group II		Group III		Group IV		Group V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	29.8	4.1	30.01	4.13	79.8	11.1	41.2	3.1	76.8	11.6
P-values										
G I vs. G II	0.992 (NS)									
G I vs. G III	<0.0001 (S)									
G I vs. G IV	0.182 (NS)									

Serum lipase	Group I (Control)		Group II		Group III		Group IV		Group V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	29.8	4.1	30.01	4.13	79.8	11.1	41.2	3.1	76.8	11.6
G I vs. G V	<0.0001 (S)									
G II vs. G III			<0.0001 (S)							
G II vs. G IV			0.191 (NS)							
G II vs. G V			<0.0001 (S)							
G III vs. G IV					<0.0001 (S)					
G III vs. G V					0.941 (NS)					
G IV vs. G V							<0.0001 (S)			

(S) significant, (NS) non-significant

Table 2: pairwise comparisons of the mean serum alpha amylase level obtained from different groups of the examined animals

Serum alpha amylase	Group I (Control)		Group II		Group III		Group IV		Group V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	50.4	8.5	51.03	8.59	106.2	15.8	63.2	4.9	104.4	12.6
P-values										
G I vs. G II	0.886 (NS)									
G I vs. G III	<0.0001 (S)									
G I vs. G IV	0.308 (NS)									
G I vs. G V	<0.0001 (S)									
G II vs. G III			<0.0001 (S)							
G II vs. G IV			0.316 (NS)							
G II vs. G V			<0.0001 (S)							
G III vs. G IV					<0.0001 (S)					
G III vs. G V					0.994 (NS)					
G IV vs. G V							<0.0001 (S)			

(S) significant, (NS) non-significant

Table 3: pairwise comparisons of the mean serum level of MDA obtained from different groups of the examined animals

Serum MDA	Group I (Control)		Group II		Group III		Group IV		Group V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	9.93	2.87	9.91	2.91	58.95	7.99	10.12	2.25	55.88	11.6
P-values										
G I vs. G II	0.985 (NS)									
G I vs. G III	<0.0001 (S)									
G I vs. G IV	0.975 (NS)									
G I vs. G V	0.004 (S)									
G II vs. G III			<0.0001 (S)							
G II vs. G IV			0.981 (NS)							
G II vs. G V			0.005 (S)							
G III vs. G IV					<0.0001 (S)					
G III vs. G V					0.883 (NS)					

G IV vs. G V							0.002 (S)		

(S) significant, (NS) non-significant

Table 4: pairwise comparisons of the mean serum level of SOD obtained from different groups of the examined animals

Serum SOD	**Group I (Control)**		**Group II**		**Group III**		**Group IV**		**Group V**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
	3.12	0.34	2.89	0.33	0.86	0.26	2.82	0.42	0.92	0.29
P-values										
G I vs. G II	0.879 (NS)									
G I vs. G III	<0.0001 (S)									
G I vs. G IV	0.697 (NS)									
G I vs. G V	<0.0001 (S)									
G II vs. G III			<0.0001 (S)							
G II vs. G IV			0.701 (NS)							
G II vs. G V			<0.0001 (S)							
G III vs. G IV					<0.0001 (S)					
G III vs. G V					0.789 (NS)					
G IV vs. G V							0.004 (S)			

(S) significant, (NS) non-significant

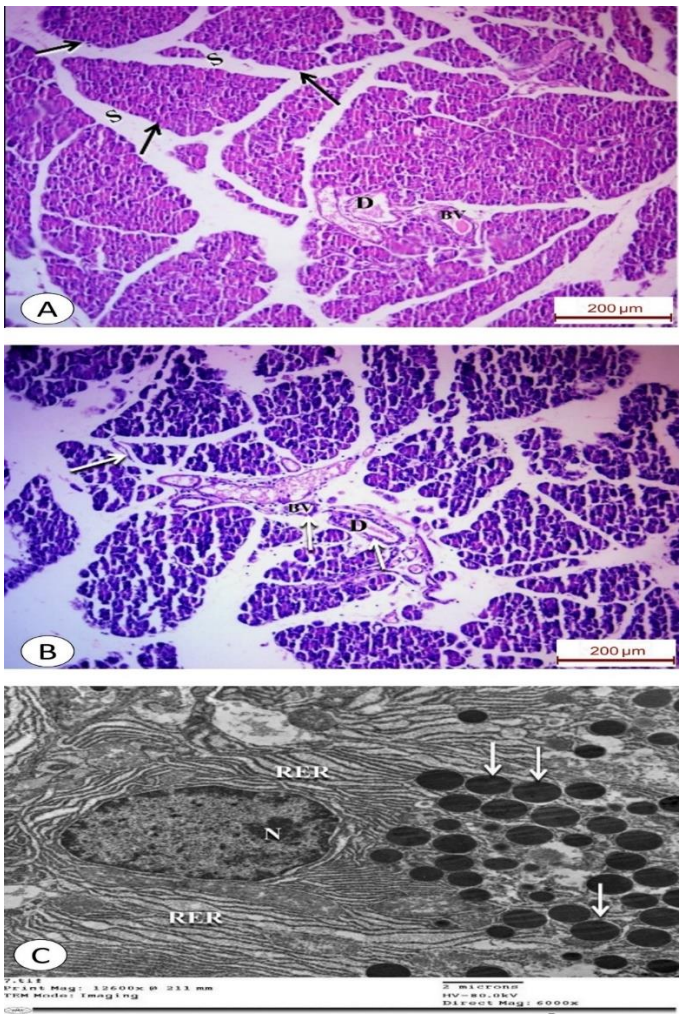

Figure 1: Photomicrographs of sections of rat pancreas from group II (sham control) showing:**A**: Normal architecture of pancreas, pancreatic exocrine acini (black arrows) arranged in lobules separated by narrow septa (S). Interlobular duct (D) and blood vessels (BV) are illustrated **(**H&E x100). **B**: Very fine connective tissue fibers (white arrows) between the acini of pancreas (black arrows), around interlobular duct (D) and blood vessels (BV) (Masson's trichrome x100). **C**: An electron micrograph showing acinar cells having an euchromatic nucleus (N), well developed cisternae of rough endoplasmic reticulum (RER) and numerous electron dense secretory granules (white arrows) (x6000).

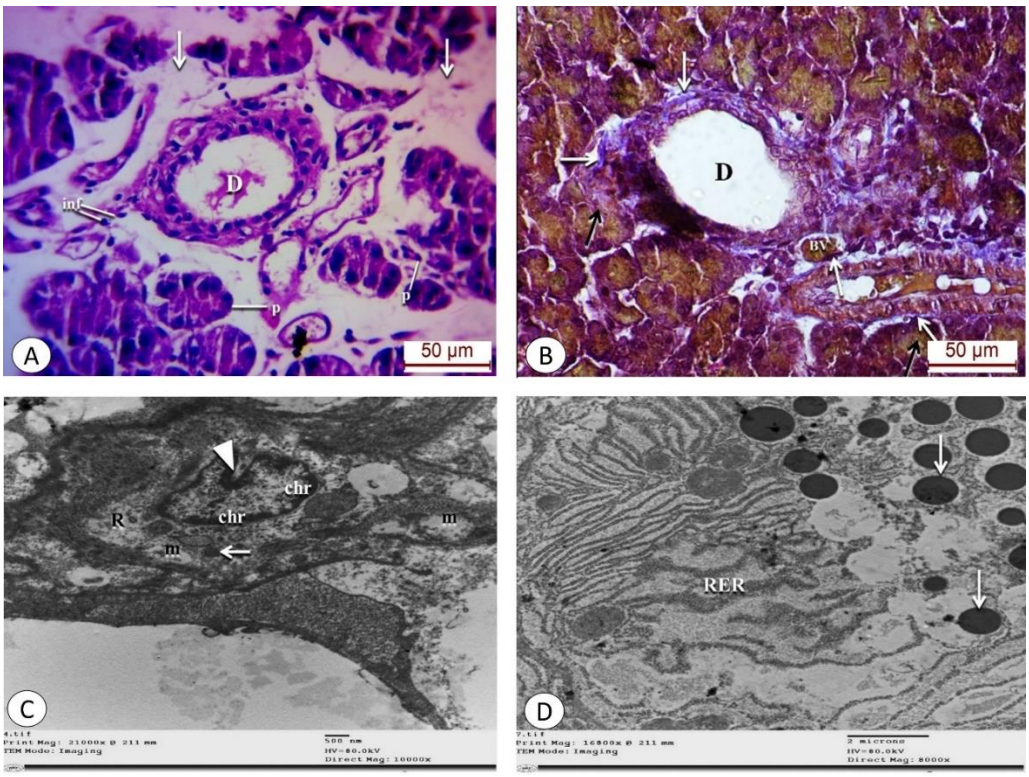

Figure 2: Photomicrographs of sections of rat pancreas from group III (ethanol-treated group) showing:**A**: Loss of architecture of pancreatic acini, widening of spaces (white arrows), dilated interlobular duct (D), inflammatory cell infiltration (inf) and pyknotic nuclei (p) (H&E x400). **B**: Excessive collagen fibers (white arrows) around pancreatic acini (black arrows), ducts (D) and blood vessel (BV) (Masson's trichrome x400). **C**: An electron micrograph showing acinar cells with indentation of nucleus (arrowhead), clumping of chromatin (chr), cytoplasmic rarefaction (R), decreased secretory granules (white arrow) and destruction of mitochondria with loss of cristae (m) (x10000). **D**: Acinar cell having dilated rough endoplasmic reticulum (RER), decreased secretory granules (white arrows) (x8000).

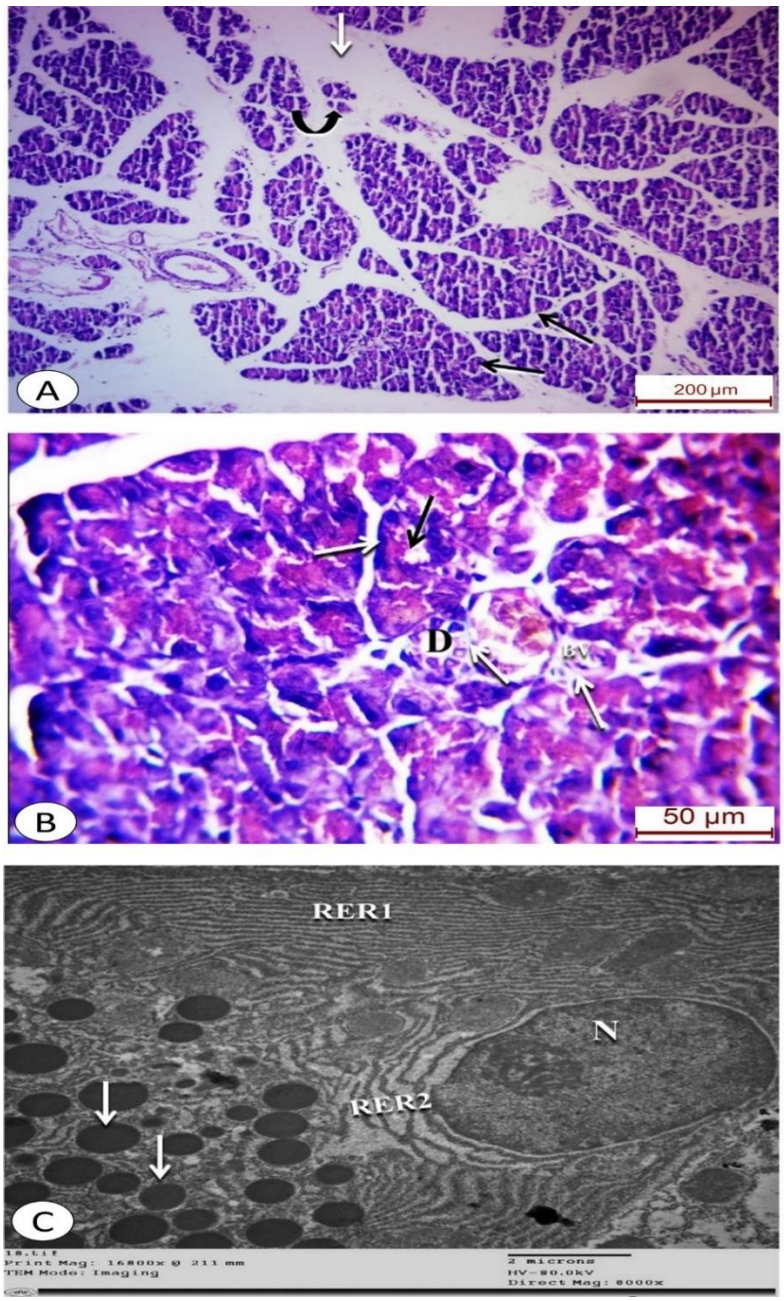

Figure 3: Photomicrographs of sections of rat pancreas from group IV (ethanol & vitamin E treated group) showing:**A**: Normal architecture of some acini (black arrows) and loss of architecture of other acini (curved arrows) and there are wide spaces between them (white arrows) (H&E x100). **B**: Minimal collagen fibers (white arrows) around acini (black arrows), duct (D) and blood vessels (BV) (Masson's trichrome x400). **C**: An electron micrograph showing acinar cells having an euchromatic nucleus (N), many secretory granules (white arrows), rough endoplasmic reticula are apparently normal (RER1) but few of them are dilated (RER2) (x 8000).

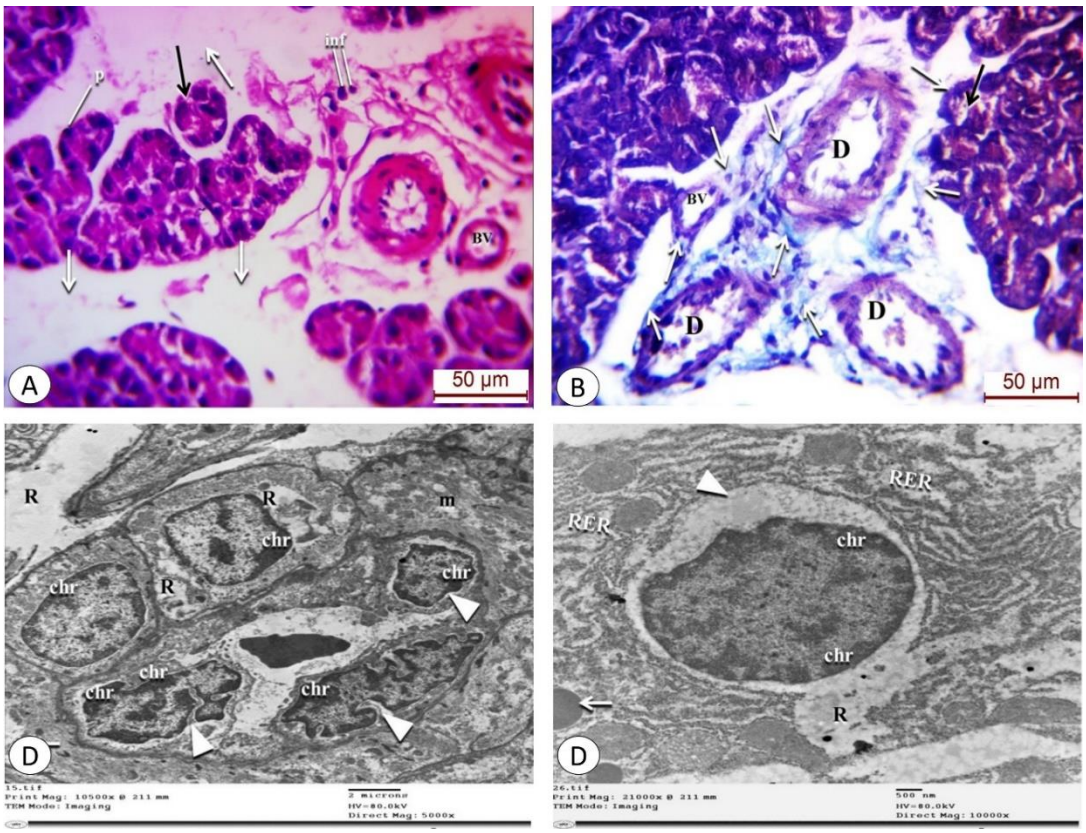

Figure 4: Photomicrographs of sections of rat pancreas from group V (withdrawal group) showing:**A**: Loss of architecture of pancreatic acini (black arrow), widening of spaces (white arrows), dilated interlobular duct (D), hypertrophied blood vessel (BV) inflammatory cell infiltration (inf) and pyknotic nuclei (H&E x400). **B**: Excessive collagen fibers (white arrows) around pancreatic acini (black arrows), dilated interlobular ducts (D) around blood vessel (BV) (Masson's trichrome x400). **C**: An electron micrograph showing acinar cells with degenerated indented nuclei (arrowhead), clumping of chromatin (chr), extensive cytoplasmic rarefaction (R), extremely decreased secretory granules (white arrow) and destruction of mitochondria with loss of cristae (m) (TEM x5000). **D**: An electron micrograph showing degenerated nucleus with clumps of chromatin (chr) and blebbing of nuclear envelop (arrowhead), dilated rough endoplasmic reticulum (RER), extremely decreased secretory granules (white arrow) (x10000).

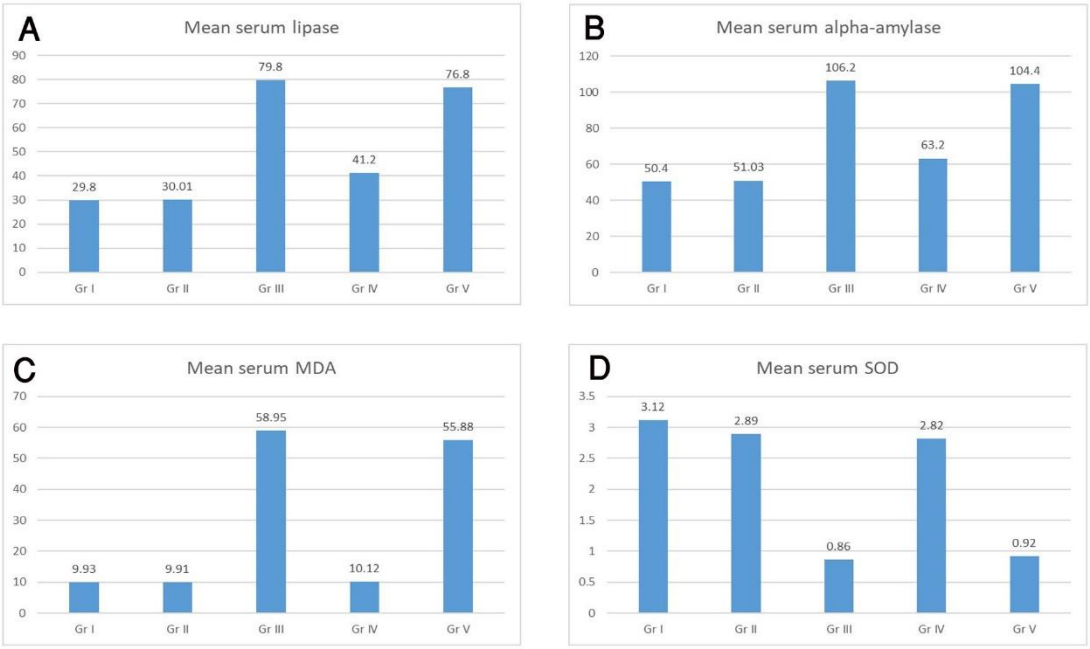

Figure 5: Histograms illustrating mean serum levels of different groups of the examined animals, **A**: Lipase, **B**: Alpha-amylase, **C**: Malondialdehyde (MDA), **D**: Superoxide dismutase (SOD).

DISCUSSION

Excessive alcohol use is a major contributor to the development of chronic pancreatitis. It is marked by significant fibrosis of the pancreatic tissue, as well as a gradual loss of pancreatic structures [1].

In the present study, loss of architecture of pancreatic acini with widening of spaces was found in group III histopathology. Similar findings were found by Lee et al. [19], who linked acinar damage to ethanol-induced oxidative stress, which causes free radical production leading to peroxidation of the cell membrane's lipid bilayer, and finally membrane disintegration.

Dilated interlobular ducts, pyknotic nuclei, and extensive deposition of collagen fibers surrounding pancreatic acini, ducts, and blood vessels were also seen in group III in the current work. This was in accordance with a previous study, which found that pancreatic alcohol-induced fibrosis was thought to be an active inflammatory process due to the production of transforming growth factor-1 and fibrosis-related cytokines from pancreatic cells [20]. Inflammation of the pancreas causes fibrosis of pancreatic ducts and the production of pancreatic stones, according to these writers. Obstruction of the main pancreatic duct may cause an abrupt aggravation of chronic pancreatitis, resulting in a necrosis-fibrosis sequence and stricture of the main pancreatic duct [20].

In the present study, collagen deposition in the pancreatic tissue was found to be significantly higher in Group III compared to Group I and II. This result agreed with Yamamoto et al. [21], who suggested that tissue fibrosis might be caused by an imbalance in tissue production and breakdown. The continuation of the alcoholic damage may result in an increase in matrix synthesis, culminating in an extracellular matrix component build-up.

The ultra-structural findings in the current study confirm the results of light microscopy. Electron microscopic examination of group III revealed indentation of nucleus, clumping of chromatin, cytoplasmic rarefaction, destruction of mitochondria with loss of cristae, dilated rough endoplasmic reticulum and decreased secretory granules. These findings were in accordance with Sadighara el al. [22], who found dilated rough endoplasmic reticulum, vacuolation, and mitochondrial destruction. The authors attributed the accumulation of dilated rough endoplasmic reticulum in acinar cells to increased protein production. Moreover, Srinivasan et al. [23] reported that chronic ethanol administration generates oxidative stress in the pancreas, which leads to mitochondrial damage with subsequent impairment in mitochondrial activity.

As compared to control and sham control groups, the oxidative marker MDA was significantly higher in group III in the present study. The antioxidant

enzyme SOD, on the other hand, was significantly lower in group III compared to control and sham control groups. This was consistent with Takahashi et al. [24], who reported that alcohol is metabolized in the pancreas mostly by oxidative mechanisms. Furthermore, Chavez et al. [25] and Vonlaufen et al. [26] found antioxidants to be effective in reducing alcohol-induced cytotoxicity.

Alcohol administration increased blood lipase and alpha-amylase levels in group III in the current work. This agreed with the findings of Kiru and Umar [27], who suggested that the greater serum amylase and lipase activity observed in rats given alcohol might be attributable to hyperplasia and the pro-inflammatory metabolic effects of alcohol. Alcohol metabolism can cause oxidative and non-oxidative pancreatic damage, which can lead to inflammatory and fibrotic processes. They further added that drinking alcohol raises the risk of pancreatitis by triggering inflammatory cascades that contribute to chronic pancreatitis.

Vitamin E is one of the most important lipid-soluble antioxidants. By scavenging intermediate peroxyl radicles, it can terminate the chain process of lipid peroxidation. It has the potential to protect the pancreas, liver, and other organs against hazardous substances [9].

Co-administration of vitamin E in group IV in this study resulted in decreased pancreatic tissue damage, according to the light microscopic findings. Similar results were reported by Gomez et al. [28], who attributed the improvement in pancreatic structure to the protective action of vitamin E, which lowers the oxidative stress generated by ethanol, thereby preserving pancreatic cells from free radical formation and increasing cellular proliferation and regeneration. In the current work, acinar cells with a euchromatic nuclei, numerous secretory granules, and intact rough endoplasmic reticulum were found in electron microscopic examination of pancreatic specimens from the same group. This was in accordance with Tong and Wang [29], who found undamaged mitochondria, Golgi apparatus, and two types of secretory granules in vitamin E-treated rats.

In the current study, few collagen fibers around acini, ducts, and blood vessels were found in Masson's trichrome-stained sections in group IV. This was consistent with the findings of Sayeda et al. [30], who found that Vitamin E was the first line of defense against the peroxidation of polyunsaturated fatty acids found in cellular and subcellular membrane phospholipids. The authors reported that vitamin E acts as an antioxidant, interrupting the chain reactions of free radicals that may be caused by hazardous chemicals in the tissues.

Vitamin E also acts as an inhibitor of oxidation processes in body tissues and was shown to protect various biomolecules, such as DNA, membrane lipids, and cytosolic proteins from oxidative damage induced by oxygen-derived free radicals [31]. Similar observations were reported in the present work with the use of vitamin E in group IV, there were a decline in MDA and an elevation of SOD. Masamune [32] pointed out that ethanol administration augments nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in the pancreatic cells producing reactive oxygen species and consequently cell damage. These injurious effects could be ameliorated by antioxidant administration assuring the role of antioxidants including vitamin E and N-acetylcysteine in inhibiting ethanol-induced injury. Vitamin E plays a leading role in controlling excessive oxidative radical formation in cellular membranes including mitochondrial membranes.

In the present work, alcohol and vitamin E administration in group IV resulted in lower blood lipase and alpha-amylase levels compared to group III. Similar results were reported by Verma et al. [31], who found that vitamin E had a protective impact on membrane stability and lipid peroxidation inhibitory effect. Due to its high lipid solubility, the vitamin wedges between the membrane phospholipids and lowers unsaturated fatty acids with 20 carbon atoms. As a result, it protects biomembranes against lipid peroxidation produced by oxygen free radicals. Moreover, alpha-tocopherol (vitamin E) is converted to alpha-tocopheryl quinone by combining with oxygen free radicals, and it undertakes the free radical scavenging process. Moreover, Jiang et al. [33] mentioned that alpha-tocopherol and tocotrienol-rich fractions reduce oxidative stress and ameliorate inflammation and fibrosis. Li et al. [34] reported that alpha-tocopherol administration elevates the survival rate, decreases fibrosis, and increases relative pancreatic weight in the chronic pancreatitis model. They further added that the serum amylase and lipase levels were statistically significantly higher, and the serum amylase and lipase levels decreased significantly statistically in the rats treated with alpha-tocopherol.

In the current work, alcohol withdrawal in group V, light microscopic examination demonstrated loss of architecture of pancreatic acini, widening of spaces, dilated interlobular duct, hypertrophied blood vessel, inflammatory cell infiltration, and pyknotic nuclei,

excessive collagen fibers around pancreatic acini and blood vessel, dilated interlobular ducts which were confirmed by electron microscopic examination as degenerated indented nucleus, clumping of chromatin, extensive cytoplasmic rarefaction, dilated rough endoplasmic reticulum, extremely decreased secretory granules with the destruction of mitochondria with loss of cristae. This agreed with the findings of Shalbueva et al. [35] who reported that during the alcohol withdrawal period, there was an increase in the production of matrix components, matrix metalloproteinases, and profibrogenic cytokines.

In the current study, alcohol withdrawal in group V could not lower levels of serum lipase and alpha-amylase. Similar results were reported by Li et al. [34], who mentioned that alcohol withdrawal did not significantly slow the progression of chronic pancreatitis. Those authors mentioned that although the serum concentration of ethanol in the abstinent rats decreased from ten-fold high to the level of normal control, neither histologic nor ultrastructural lesions in acinar cells induced by chronic ethanol administration could be reversed. They added that exocrine pancreatic insufficiency in alcoholic rats could not be reversed following alcohol withdrawal which might be attributed to the profound injury of the pancreas.

CONCLUSIONS

Ethanol administration is a leading cause of exocrine pancreatic damage due to its inflammatory role and increased oxidative stress, withdrawal from alcohol could not overcome these harmful effects. However, concomitant administration of vitamin E with ethanol could significantly ameliorate this damage.

RECOMMENDATIONS

Further studies are needed to evaluate the effect of ethanol administration on the endocrinal part of the pancreas. Withdrawal period from ethanol in the current work could not overcome pancreatic damage, therefore, concomitant administration of vitamin E with ethanol could be beneficial for alcohol users.

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Conflicts of interest

There are no conflicts of interest.

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