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Original article

Protective effect of vitamin E against ethanol-induced small intestine damage in rats

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ABSTRACT

The role of oxidative stress and inflammatory reaction has been reported in various ethanol-induced complications. The purpose of this study was to evaluate the effect of ethanol-induced structural alteration, oxidative stress, and inflammatory reaction on the small intestine of rats, and plausible protective effect of vitamin E to determine whether it inhibits the abnormality induced by ethanol in the small intestine. Twenty-four male wistar rats were divided into three groups, namely: Control©, ethanol, and vitamin E treated ethanol groups.

After six weeks of treatment, the small intestine length, villus height, crypt depth and muscular layer thickness, oxidative stress, and inflammatory parameters showed significant changes in the ethanol treated group compared to the control group. Vitamin E consumption along with ethanol ameliorated structural alteration of the small intestine and reduced the elevated amount of oxidative stress and inflammatory markers such as protein carbonyl, OX-LDL, IL-6, Hcy, and TNF-α. Furthermore, their total antioxidant capacity was increased significantly compared to that of the ethanol group. These findings indicate that ethanol induces the small intestine abnormality by oxidative and inflammatory stress, and that these effects can be alleviated by using vitamin E as an antioxidant and anti-inflammatory molecule.

1. Introduction

Results from a number of studies indicate that chronic ethanol ingestion increases the risk of gastrointestinal diseases. More recent studies have documented that exposure of small intestine to ethanol causes a variety of structural and functional abnormalities in the small intestine [1,2]. As a functional feature, ethanol exposure decreases the absorption of D-glucose, D-xylose, L-amino acid residues, monoglycerides, fatty acids, and vitamins like vitamin A and folic acid. it has also been reported by previous studies that alcohol exposure is associated with zinc, Iron and manganese deficiencies [3].

From a morphological perspective, ethanol intake reduced villus cell population in rats and inhibited their crypt cell proliferation. In addition, acute administration of ethanol led to mucosal damage in the small intestine exhibited as a loss of epithelium at the tips of the villi, haemorrahagic erosions, and haemorrhage in the lamina. Moreover, changes in the gastric motility, such as delayed emptying of solid food from the stomach increased the mean number of phasic contractions in the duodenum and villus. Furthermore, it impaired tight junction proteins among epithelial cells. This has been reported as a result of ethanol consumption by previous studies [2,4–6]. Despite finding different abnormalities in the ethanol-exposed small intestine, the precise mechanism underlying ethanol-induced small intestine structural and functional changes has not yet been completely clarified. Although earlier studies have implied that ethanol directly imposes toxic effects on the small intestine [1], a growing body of evidence from recent studies indicates that ethanol causes tissue damage via indirect pathways. Our recent works and others' have confirmed that ethanol administration in male rats and pregnant female rats results in oxidative stress and inflammatory changes in the heart, aorta, and small intestine. These changes are evidenced by increased protein oxidation, lipid peroxidation, and pro-inflammatory cytokines [7–9]. Since such cytokines and oxidative stress parameters have deleterious effects on different organs, it seems possible that the harmful effects of

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ethanol exposure could be due, at least in part, to the oxidative stress and inflammatory reaction. Moreover, the beneficial effects of antioxidant therapy during alcohol exposure support the idea that ethanol may exert its deleterious effects mainly via oxidative stress [8,9]. Based on the mentioned evidence, we propose that the deleterious effect of chronic ethanol consumption on the small intestine is entirely or partially mediated by oxidative stress and inflammatory reaction. To test this hypothesis, the possible protective effects of vitamin E against ethanol-induced morphological alteration, oxidative stress, and inflammatory reaction were also investigated in the small intestine of male rats.

2. Materials and methods

All experimental procedures described herein were performed in accordance with the Principles of Laboratory Animal Care (NIH publication, no.85–23, revised 1985) and were approved by the Urmia University of Medical Sciences Animal Care Committee.

Twenty-four adult male Wistar rats weighing 220 ± 20 g were assigned to three groups ($n = 8$ in each group), namely: 1 control, 2 ethanol treated, and 3 ethanol–vitamin E treated groups. Ethanol-treated rats received 4.5 g/kg body weight ethanol (Merck KGaA, Darmstadt, Germany) solution in tap water (20% w/v) intragastrically by gavage once per day for six weeks. According to our preliminary studies, rats in the ethanol–vitamin E group received a nontoxic dose of 300 mg of vitamin E (Merck GmbH, Germany) intragastrically by gavage in addition to their regular daily diet and the same amount of ethanol. The control group was treated with vehicle only (tap water).

Food and water were supplied ad libitum to all groups throughout the experiment. After six weeks, all rats were anesthetized using 10% chloral hydrate (0.5 ml/kg body weight) intraperitoneally, and anesthesia was assessed through pinching a hind paw. In order to empty the small intestine, all animals were fasted for 12 h. After weighing, the abdominal cavity was opened, and the small intestine was cut between pyloric and ileocecal sphincters. Excised small intestine was freed from adventitial tissues, fat, and mesentery. The length and weight of the small intestine were measured. The samples five cm in length were taken from three segments of the small intestine namely duodenum, jejunum, and ileum and were kept in 10% formalin buffer for 72 h. For biochemical analyses, the remaining part of duodenum was segmented, each segment was flushed with chilled 115 g/L KCL solution, and the mucosa was scraped (11). A 20% weight/volume ice cold homogenate was prepared in 50 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 × g for 20 min at 4 °C in a refrigerated centrifuge (HERMLE, Z 360 K, Germany). The supernatant was collected and stored at −80 C until the time of analysis.

2.1. Biochemical assays

2.1.1. Total antioxidant capacity

Measuring the amount of total antioxidant capacity in rat's small intestine tissue was carried out by using an antioxidant assay kit (Cayman chemical, USA). Aqueous and lipid soluble antioxidants were not separated in this protocol, thus the combined antioxidant activities of all its constituents including vitamins, proteins, lipids, glutathions, uric acid, etc. were assessed. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS•+ by metmyoglobin and measures all aqueous- and lipid-soluble antioxidants including vitamins, proteins, lipids, glutathione, uric acid etc. The capacity of the antioxidants in the sample, in terms of prevention of ABTS oxidation, was compared with that of Trolox, a water-soluble tocopherol analogue, and was quantified as millimolar Trolox equivalent. The results were normalized by total protein content of each tissue sample. Detection range of the kit was 0.044 – 0.330 mM.

2.1.2. Protein carbonyl

Protein carbonyl content was measured in the supernatant samples using a protein carbonyl assay kit (Cayman chemical, USA). This kit utilizes the 2,4-dinitrophenylhydrazine (DNPH) reaction to measure the protein carbonyl contents in tissue homogenates or plasma and serum in a convenient 96-well format. Briefly, DNPH reacts with protein carbonyl forming a Schiff base to produce the corresponding hydrazone, which can be analyzed spectrophotometrically at an absorbance level between 360–385 nm. The carbonyl content was then standardized to the protein concentration.

2.1.3. Hcy

Homocysteine (Hcy) levels were measured using an Axis Shield kit (Axis Shield, UK). The principle of using the assay is as follow: bound or oxidized form of homocystein(Hcy) is reduced to free Hcy. Free hcy then reacts with serine by cystathionine beta-syntase and forms cystathionine. Cystathionine breaks down by cystathionine beta-lyase and forms Hcy, pyruvate, and ammonia. Pyruvate then is converted to lactate by lactate dehydrogenase with nicotinamide adenine dinucleotide(NADH) as coenzyme. The rate of NADH conversion to NAD+ is in direct proportin to the concentration of homocysteine (Δ A340 nm).

2.1.4. IL-6

The concentration of IL-6 in the rats' small intestines was measured by a standard sandwich enzyme-linked Immunesorbent assay kit (EK0412, Boster Biological Technology Ltd., USA). All steps were performed as described in the protocol provided by the manufacturer. The optical densities were read at 450 nm and the concentration of the samples was calculated using the standard curve as pg/ml IL-6. The minimum detectable dose (lower limit of detection) and the detection level of rats' IL-6 were typically less than 5 pg/ml and 62.5–4000 pg/ml, respectively.

2.1.5. TNF-α

TNF-α was assayed using a double-sandwich elisa technique and the ELISA kit (Hangzhou, Eastbiopharma, China). Briefly, two antibodies used in this method were monoclonal antibody (precoated antibody) and detecting antibody (polyclonal antibody) labeled as biotin. Samples and biotin labeling antibody were added into the ELISA plate wells and washed out with PBS. Then, Avidin-peroxidase conjugates were added to ELISA wells in order; TMB substrate was used for coloring after the reactant was thoroughly washed out by PBS. TMB turned into blue in peroxidase catalytic and finally turned into yellow under the action of acid. The color depth and testing factors in samples were positively correlated.

2.1.6. Ox-LDL

Tissue Ox-LDL levels were measured using a capture ELISA (also known as a "sandwich" ELISA) kit, in which the wells of the microtiter plates were coated with the capture antibody mAb-4E6 (Mercodia, Sweden). Diluted supernatant samples (1:6561) were used for ELISA measurements. The optical density of the wells was read at 450 nm, and the results were calculated.

2.2. Histological examinations

After tissue processing steps and preparation of paraffin blocks, a series of 5 μm thick sections of duodenum, jejunum, and ileum tissues were stained deploying the hematoxylin-eosin method. The sections were examined using a research microscope equipped with a digital camera (Olympus, CX 31, Japan). Histological

parameters quantification of microscopic components such as villus height, crypt depth, muscular layer thickness, submucosal layer, and total small intestine wall thickness were carried out using a linear scale-ocular micrometer providing a 2.5 μm interval between divisions under 400 × magnifications a (Fig. 1). Eight sections of the duodenum, jejunum, and ileum tissues from different groups were assessed and the results were expressed as mean ± SE.

In each section, any number of intended parameters, such as villus height, crypt depth, etc.,which were present in four or five fields, were measured and the mean of data on all of them was considered as a datum for each section. Therefore, eight mean data were obtained for each parameter.

All morphometric measurements were carried out by at least three independent expert examiners in a blinded manner and expressed in comparison to controls.

2.3. Statistical analysis

Statistical differences between the groups were tested applying one-way ANOVA SPSS 16 software, followed by Tukey's post hoc test. In each test, the data are expressed as the mean ± S.E., and $p < 0.05$ is considered statistically significant.

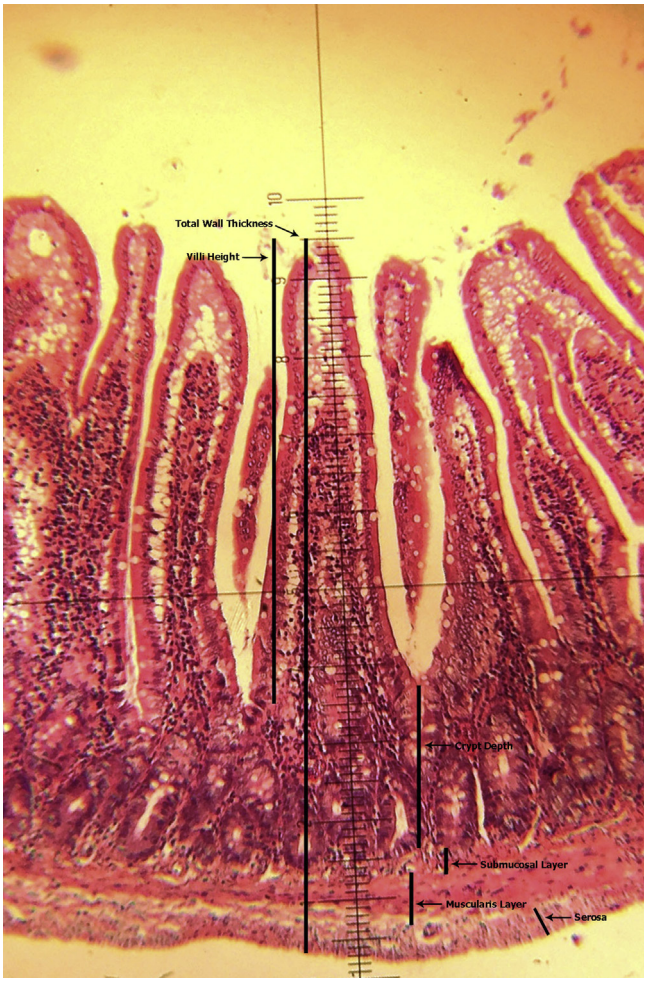

Fig. 1. Histological parameters quantification of microscopic components such as villus height, crypt depth, muscular layer thickness, submucosal layer, and total small intestine wall thickness were carried out using a linear scale-ocular micrometer providing a 2.5 μm interval between divisions under 400 × magnifications.

Table 1
Effect of vitamin e on ethanol-induced changes in small intestine weight, length, oxidative stress parameters, and inflammatory cytokines.

	Control	Ethanol	Ethanol + vit E
small intestine length (cm)	106.5 ± 2.28	116 ± 2[a]	102 ± 1.9[a,b]
small intestine weight (g)	5 ± 0.34	5.29 ± 0.17	5.5 ± 0.12
weight (mg)/length (cm)	49.57 ± 2	45.6 ± 1.6	50 ± 1.8
Total antioxidant capacity (mM)	0.04 ± 0.002	0.02 ± 0.009[a]	0.13 ± 0.012[a,b]
Protein carbonyl (nmol/mg)	8.01 ± 0.63	26.2 ± 1.33[a]	14.28 ± 0.64[a,b]
Ox-LDL (IU/L)	13.83 ± 0.6	18 ± 1.19[a]	18.16 ± 1.6[a]
Hcy (mg/L)	4.28 ± 0.1	6.43 ± 0.25[a]	4.63 ± 0.13[b]
IL-6 (ng/ml)	153 ± 7.4	318.83 ± 24.6[a]	209.83 ± 14.6[a,b]
TNF-α (Pg/ml)	105.13 ± 9.4	131.28 ± 0.84[a]	119.27 ± 4.6[b]

Values are mean ± SE for 8 rats per group.
[a] Denotes significant difference ($p < 0.05$) compared to the control.
[b] Denotes significant difference ($p < 0.05$) compared to the ethanol treated group.

3. Results

Table 1 presents the mean values of oxidative stress and inflammatory cytokines constituent levels in rats' small intestine tissue in different groups under study. The total antioxidant capacity showed a significant decrease in the ethanol group compared to the control group ($p < 0.05$). Vitamin E administration along with ethanol, largely enhanced the total antioxidant capacity compared to ethanol and control groups ($p < 0.05$).

The protein carbonyl level was increased in the ethanol group compared to that in the control group ($p < 0.05$). The level of protein carbonyl in small intestine tissue was significantly decreased in the vitamin E-treated rats ($p < 0.05$), but it was still significantly higher than that in the control group ($p < 0.05$). As shown in Table 1, the level of Ox-LDL was significantly increased in the small intestine of both the ethanol and ethanol–vitamin E treated group in comparison to the control group ($p < 0.05$). There was no significant difference between the ethanol and ethanol–vitamin E treated group ($p > 0.05$). Hcy levels in the small intestinal tissue of the ethanol-treated group were significantly higher than those in the control group ($p < 0.05$). There were no significant differences between ethanol–vitamin E treated and the control groups regarding Hcy levels of the small intestine tissue ($p < 0.5$). The results shown in Table 1 indicated that treatment of animals with ethanol increased IL-6 level of small intestine tissue significantly compared to those in the control group($p < 0.05$). Vitamin E administration along with ethanol decreased the elevated level of IL-6 compared with those exhibited by the control group ($p < 0.1$). Similarly, TNF-α level was significantly elevated in small intestine tissue obtained from ethanol group compared to the one obtained from the control group ($p < 0.05$), but no significant differences were detected between the vitamin E treated and the control group ($p < 0.3$).

The mean value of small intestine length was 106.5 ± 2.28 cm, 116 ±2 cm, and 102 ± 1.9 cm in the control, ethanol, and ethanol-vitamin E groups, respectively. Small intestine length were significantly increased in the ethanol group compared with that in the control group ($p < 0.05$). There were no significant differences between ethanol-vitamin E treated and control groups. The small intestine weight was not significantly changed among the control, ethanol, and ethanol-vitamin E treated groups. Similarly, the small intestine weight (mg)/small intestine length (cm) ratio did not show significant differences among different groups.

Table 2 and Fig. 1 show the morphological changes in the duodenum, jejunum, and ileum of the small intestine in all groups. The small intestine wall thickness in the duodenum of ethanol group showed a significant increase compared to that in the control group ($p < 0.05$). Although vitamin E administration along

Table 2
Morphological changes of small intestine in control, ethanol and ethanol–vitamin E treated groups.

	Groups	Duodenum	Jejunum	ileum
Villi Height (μ)	Control	53.2 ± .7	46 ± 1.3	44 ± 1.3
	Ethanol	54 ± 1.4	23.9 ± 1[a]	22.4 ± 2.3[a]
	Ethanol + vit E[a,b]	37.2 ± 1[a]	43.6 ± 3[b]	31 ± 1.9[a,b]
Crypt depth (μ)	Control	12 ± .4	9 ± .3	10 ± .8
	Ethanol	32.2 ± 1[a]	17.6 ± .1[a]	22.5 ± 1.7[a]
	Ethanol + vit E	39.3 ± .8[a,b]	23.5 ± 1.2[a,b]	18 ± .4[a,b]
Submucosal layer (μ)	Control	5.6 ± .5	4.3 ± .2	5.4 ± .3
	Ethanol	7.1 ± .5[a]	3.6 ± .4	7.5 ± .9[a]
	Ethanol + vit E	7 ± .2[a]	5.6 ± .2[a,b]	4.35 ± .2[b]
Muscle layer (μ)	Control	7.8 ± .5	3.3 ± .5	4.5 ± .34
	Ethanol	10 ± .6[a]	2.9 ± .1	4.7 ± .2
	Ethanol + vit E	4.3 ± .1[a,b]	3.4 ± .3	3.2 ± .2[a,b]
Serosa layer (μ)	Control	4.6 ± .4	3.5 ± .2	3.6 ± .2
	Ethanol	3.6 ± .3[a]	2.3 ± .2[a]	4.2 ± .1[a]
	Ethanol + vit E	3.6 ± .3[a]	3.6 ± .1[b]	2.7 ± .2[a,b]
Total wall thickness (μ)	Control	83.8 ± 1.7	62.6 ± 2.3	63.8 ± 1.2
	Ethanol	103 ± 2.9[a]	51 ± .8[a]	58.1 ± 1.14[a]
	Ethanol + vit E	93.5 ± 1.4[a,b]	82.7 ± 1.9[a,b]	58.6 ± 1.5[a]

Values are mean ± SE for 8 rats per group.
[a] Denotes significant difference ($p < 0.05$) compared to the control.
[b] Denotes significant difference ($p < 0.05$) compared to the ethanol treated group.

with ethanol reduced small intestine wall thickness compared to that observed in the ethanol group, it was still higher than that of the control group ($p < 0.05$). In the jejunum and ileum, small intestine wall thickness significantly decreased in alcohol treated rats ($p < 0.05$). The thickness of small intestine wall in jejunum of vitamin E treated group, were increased significantly compared to that in the ethanol and control groups ($p < 0.05$). In the ileum, there were no significant differences between the vitamin E treated group and ethanol group. In ethanol treated rats, villus height in duodenum showed no significant change compared to that in the control group ($p < 0.1$). Vitamin E consumption decreased villus height significantly compared to that of the ethanol and control group ($p < 0.05$). In the jejunum and ileum part of small intestine, the villus height showed significant reduction compared to that in the control group ($p < 0.05$). Vitamin E administration increased villus height significantly compared to that of the ethanol group ($p < 0.05$), but it was still significantly lower than that of the control group ($p < 0.05$). As shown in Table 2, the crypt depth in three segments of the small intestine markedly increased in the ethanol group as well as in the vitamin E treated group compared to that in the control group ($p < 0.05$). Crypt depth increase in the vitamin E treated group was more pronounced than that in the ethanol group.

The smooth muscle layer thickness of small intestine showed a significant increase in the duodenum of ethanol group compared to that of the control group ($p < 0.05$). Vitamin E treatment significantly reduced the smooth muscle layer thickness compared to ethanol treatment. In the jejunum and ileum segment of the small intestine, there were no significant differences in terms of smooth muscle layer thickness among different groups.

4. Discussion

In the present investigation it was demonstrated that six weeks of ethanol consumption caused the following; (1) Occurrence of oxidative stress and inflammatory reaction in the small intestine of rats manifested by the increased protein carbonyl, Ox-LDL, Hcy, TNF-α, and IL-6, as well as the decreased total antioxidant capacity; (2) Morphological changes, such as the villus height, crypt depth, smooth muscle layer thickness, as well as total small intestine wall thickness were also found in the small intestine wall. Moreover, these alterations were more pronounced in the duodenum segment of the small intestine than in the jejunum and ileum segments; and (3) Vitamin E administration concurrent with ethanol, significantly ameliorated morphological alteration of the small intestine and improved oxidative stress and inflammatory reaction, compared to that of the ethanol group. It was previously shown, by using different experimental systems in animals and humans, that chronic and acute ethanol exposure result in structural and functional changes in the small intestine.

Although previous studies have documented some measures of functional and structural alterations induced by ethanol in the small intestine (mentioned in the introduction), the underlying mechanism through which ethanol exerts its deleterious effects on the small intestine has not been completely clarified. In the current study, the data clearly show occurrence of oxidative stress and inflammatory reaction in the small intestine, and that this is temporally correlated with morphologically significant alterations in the small intestine structure. High concentrations of ethanol after oral ingestion only occur in the upper small intestine, that is, in the duodenum. This concentration results in severe structural changes such as intestinal wall thickness, villus height, and crypt depth which are predominantly in the duodenum segment as opposed to the jejunum and ileum segments [2]. Our previous studies and others have indicated that the generation of free radicals during the course of ethanol oxidation and a subsequent increase in lipid peroxidation plays a major role in ethanol induced brain, heart, and hepatic injury [10,11]. The results of the current study showed that ethanol consumption significantly increased the protein carbonyl and Ox-LDL level in the small intestine tissue of rats. The increase in protein carbonyl and Ox-LDL level observed in this study is consistent with our recent similar findings in hippocampus and aorta tissue of ethanol-exposed rats and rules out its involvement in raising the ROS level [12,13]. The increase in the protein carbonyl content is an indication of both oxidative change and chemical modification of proteins that may lead to structural alteration and functional inactivation of many enzymes [14]. In addition, we have shown that the Hcy level in the small intestine tissue increases as a result of exposure to ethanol. The sulfur-containing amino acid Hcy increases free radical generation by various means including the oxidation of its solfhydryl group, activation of NADPH oxidases, enhancement of protein oxidation, as well as weakening of the antioxidant defense system [15,16]. In addition, the Hcy induces expressions of many pro-inflammatory factors such as IL-6, TNF-α, and causes denaturation of key molecules by hypomethylation of DNA and proteins [17,18].

Interestingly, in the present study, Hcy concentration in small intestine tissue was increased due to ethanol consumption. This increase was accompanied by the significant increase of protein oxidation, lipid peroxidation, IL-6, and TNF- α and the decrease of total antioxidant capacity. The study conducted by Hao-Ding et al., indicated that the Hcy and free radicals promote expression of matrix metalloproteinases in the intestinal wall [19]. These proteolytic enzymes play an important role in regulating the metabolism of the extracellular matrix. Accordingly, ethanol-induced free radical generation and enhancement of Hcy in the small intestine wall may destroy the intact intestinal mucosal structure and increase mucosal permeability of small intestine through matrix metalloproteinases enzymes [20].

Another result of the current study is the significant increase of small intestine length without a change in the small intestine weight and small intestine weight/length ratio in the ethanol group compared to the control group. This is probably an indication of a reduction in the small intestine smooth muscle layer tonicity due to ethanol exposure. Previous studies have indicated that ethanol exposure leads to relaxation of urinary bladder smooth muscle cells with enhancing of ca^{2+} activated K^{+} (BK) channel activity and inhibiting L-type Ca^{2+} in guinea pigs [21]. In addition, moderate doses of ethanol decreased contractility of antrum and duodenum smooth muscles in the rats[22].The results of these studies and our study provide evidence that many ethanol-induced abnormalities in gastrointestinal are plausibly mediated by interference of ethanol with K^{+} and Ca^{2+} activity. The protective effect of vitamin E on the small intestine morphological alteration, inflammatory responses, and oxidative stress induced by ethanol in the small intestine tissue was the second subject addressed in the current study. In earlier studies, we have shown that vitamin E administration has a supportive feature and protective effect against ethanol-induced abnormalities in the heart, testis, and brain [9,11,23]. In the current study, however, we found it is possible that attenuation of oxidative stress and inflammatory reaction mediates the protective effect of vitamin E against ethanol-induced small intestine abnormalities. Vitamin E is a major scavenger of free radicals, and this ability permits it to eliminate lipid soluble free radicals. Furthermore, it supports several intracellular enzymatic antioxidant defenses including catalase, SOD, and other enzymes, as well as small organic molecules such as L-ascorbic acid [24]. Vitamin E has also been shown to have intrinsic anti-inflammatory properties and to suppress pro-inflammatory cytokines expressions such as TNF-α, COX-2, and IL-6 in chronic ethanol exposure on rat pancreas [25]. In addition, vitamin E inhibits expression of VCAM-1 and ICAM-1 in endothelial cells. It also reduces plasma serine protease generation in ethanol-exposed rats and in the diabetics [25–27]. Accordingly, if ethanol consumption induces some functional and structural abnormality through oxidative stress and inflammatory reaction as confirmed by previous studies, the rescue effect of vitamin E on these abnormalities may be due to vitamin E antioxidant and anti-inflammatory properties. Drawing from the results of current study and the previous works, we suggest that ethanol-induced oxidative stress and inflammatory reaction may play a major role in small intestine abnormalities resulting from ethanol consumption.

In conclusion, there are several notable findings in this study. Rats exposed to ethanol showed greater protein carbonyl, higher level of Ox-LDL, Hcy, IL-6 and TNF-α, as well as a lower level of total antioxidant capacity in the small intestine tissue. In addition, we observed evidences of the alterations in the small intestine structure such as increased villus height, crypt depth, smooth muscle layer thickness. These alterations, compared to jejunum and ileum segments, were more pronounced in the duodenum. Furthermore, vitamin E administration alleviated structural changes, restored inflammatory cytokines, and oxidative stress parameters in the experimental group compared to the control group. The elevated level of oxidative stress and inflammatory cytokines in one hand, and the restorative effects of vitamin E against these alterations on the other hand, strongly support the hypothesis that ethanol-related alterations in the small intestine structure are caused by oxidative stress along with inflammatory responses. Further investigations are needed to elucidate exact molecular mechanisms by which ethanol exerts its deleterious effects on small intestine via oxidative stress and inflammatory reaction.

Conflict of interest

None.

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