Indian Journal of Biochemistry & Biophysics
Vol. 61, September 2024, pp. 517-526
DOI: 10.56042/ijbb.v61i9.11341

Assessing the protective effects of chard on valproic acid-induced pancreatic complications

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Received 24 May 2024; *revised 11 June 2024*

Valproic acid (VPA) treatment is known to potentially cause adverse effects, notably as the most common cause of drug-induced acute pancreatitis. It is crucial to balance the therapeutic benefits and potential major side effects of VPA administration. Complications associated with VPA may arise from toxic VPA metabolites and alterations in antioxidant levels. While chard is well-known for its anti-hyperglycemic and antioxidant properties, there is a lack of research on its impact on the pancreas during VPA treatment. This study aimed to explore the possible protective effects of chard against VPA-induced complications in the pancreas using histological and biochemical approaches. Animals were separated into four groups: i) Control, ii) received chard (100 mg/kg), iii) received VPA (500 mg/kg), and iv) received VPA+Chard (in the same dosages and time). On the eighth day, the rats' pancreatic tissue and blood specimens were collected. In the Chard and VPA+Chard groups, chard decreased blood glucose levels compared to the control and VPA groups. In comparison to the VPA group, the VPA+Chard group pancreatic glutathione level and catalase activity increased whereas malondialdehyde levels decreased. Furthermore, administration of chard to the control and VPA groups increased tissue factor activity and sialic acid level as compared to the VPA group. The histological findings confirmed the biochemical results. It is therefore concluded that chard has the potential to protect pancreatic tissue from VPA-induced complications by reducing lipid peroxidation and blood glucose while enhancing antioxidants and sialic acid levels.

Keywords: Antiepileptic drug, Antioxidant, *Beta vulgaris* L.var. *cicla*, Oxidative stress, Pancreas

The pancreas, a glandular organ, has a dual functions as both exocrine and endocrine. It plays essential role(s) in the digestion of food and in regulating the utilization of glucose for energy following the digestion process. While various tissues play roles, the regulation of glucose levels in the blood within a narrow physiological range is primarily controlled by the pancreas and brain[1]. Damage to the pancreatic cells could affect both endocrine (*i.e.*, production of hormones that control blood glucose levels and glandular secretion) and exocrine (*i.e.*, production of digestive enzymes) functions of the pancreas. Pancreatic disorders are frequently connected to abdominal pain, malnutrition, complications, and a diminished quality-of-life. Also, diabetes arising as a result of pancreatic disease holds potential significance[2].

Valproic acid (VPA) is employed in the treatment of seizures, bipolar disorder, and certain types of headaches. Apart from these positive effects, VPA has potential adverse effects including hepatotoxicity and teratogenicity[3]. It is also the most common reason for drug-induced acute pancreatitis[4]. However, it is unclear how VPA damages the pancreas. The VPA-induced complications might be caused by toxic VPA metabolites and changes in antioxidants. Hepatotoxicity is primarily caused by two of the VPA metabolites, 2, 4-diene-VPA and 4-ene-VPA[5]. Oxidative stress due to unbalanced oxidant and antioxidant status can be harmful to all tissues.

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It has been long postulated that it is necessary to balance the over-production of free radicals with antioxidants, which are produced endogenously or supplied in the diet. Antioxidants prevent the formation of free radicals, and also detoxify and scavenge them. Fresh fruit and vegetables are natural sources of antioxidants, including vitamins, polyphenols, and trace elements. There are some peer-reviewed studies in which both natural and synthetic antioxidants such as green tea and quercetin are reported to protect the pancreas from the harmful effects of free oxygen radicals in experimental animals and patients with acute pancreatitis[6,7].

Chard, is known as *Beta vulgaris* L. var. *cicla*, is extensively utilized both as a culinary ingredient and as a medicinal herb. Edible components of the plant are leaves and stems. The pharmacological activities of chard extracts are antioxidant, antitumor, hemostatic, and hepatoprotective effects additionally anti-acetylcholinesterase, hypoglycemic and anti-inflammatory effects[8-11]. These effects are due to its vitamins such as C and E, flavonoids, phenolics, saponins, phospholipids, glycolipids, minerals, carotenoids, fatty acids, and folic acid content[12-14]. Previous studies have demonstrated the beneficial effects of vitamin E[15], vitamin C[16], folic acid[17] and plant-derived flavonoids/phenolics such as quercetin[18], caffeic acid[19], and apigenin[20] in experimental cases of pancreatitis. Therefore, using chard in the treatment of VPA may be effective in avoiding tissue damage of pancrease.

Studies indicate the possibility of VPA treatment-related adverse effects, and it is critical to maintain a balance between effective therapeutic possibilities and major adverse reactions following VPA administration to promote the beneficial effects of VPA. Chard is mainly characterized as an anti-hyperglycemic and antioxidant; however, the effects of chard administration on histological and biochemical parameters in the case of VPA treatment have not been reported in the pancreas. Therefore, the objective of this study was to investigate the potential preventive effects of chard extract on VPA complications.

Materials and Methods

The aqueous chard extract preparation

The chard leaves were obtained from the markets in Istanbul, Türkiye and identified by the member of Istanbul University Faculty of Pharmacy, Prof. Dr. Kerim Alpınar (Voucher specimen number: 67901). All experimental proceedings were allowed by the Marmara University Animal Care and Use Committee (70.2014.mar). The chard leaves were washed in distilled water before being dried at room temperature (25°C). 100 g of dried chard leaves were extracted by boiling with 1 L of distilled water for 8 h until the boiling point of the water. After the extract was filtered, its water was removed from the rotary evaporator. Chard extract was weighed and administered to rats at a dose of 100 mg/kg. The weighed extract was dissolved in 1 mL of distilled water. The extract was given to rats by gavage for 7 days[21].

Animals

Thirty-two female Sprague Dawley rats were used in the study. They were maintained under controlled conditions with a 12 h shifts in light-dark cycle, at 25±2°C and at 55±8% relative humidity range in the laboratory. The rats were fed with standard pellets and provided water *ad libitum*. The Marmara University Animal Care and Use Committee has approved the study with the decision number of Ethics Committee as 70.2014.mar.

Experimental design

There were one control (C) group and three experimental groups; *i.e.*, chard group (C+Chard), valproic acid group (VPA) and valproic acid +chard group (VPA+Chard). In the chard groups, chard extract was given as 100 mg/kg/day by gavage for 7 days. It was administered one hour before VPA administration. In the valproic acid groups, VPA was given as 500 mg/kg/day intraperitoneally for 7 days. Both at the start and the end of the experiment, body weight was recorded. Under ether anaesthesia, on the eighth day of the experiment, the rats were sacrificed, and tissue samples from the pancreas as well as blood specimens were collected. One-half of the pancreatic tissue samples were used for histological examinations after fixation with 10% formaldehyde. The remaining half of the tissue samples were weighed, and then homogenized in 0.9% of sodium chloride solution. This homogenate (10% *w/v*), stored at -20°C, was subsequently used for biochemical analyses.

Estimation of blood glucose level

Blood glucose levels after an 18 h fasting were measured at the end of the experiment using the *o*-toluidine method[22].

Quantification of oxidant-antioxidant status and sialic acid

In pancreas tissue homogenates, reduced glutathione (GSH) was estimated by Ellman's

reagent[23]. The findings were given as mg GSH/g protein. Lipid peroxidation (LPO) levels were assayed spectrophotometrically at 532 nm using with thiobarbituric acid reaction[24]. The end product of LPO is malondialdehyde (MDA). The findings were given as nmol MDA/mg protein. Superoxide dismutase (SOD) activity was determined based on its ability to enhance the riboflavin-sensitized photooxidation of *o*-dianisidine[25]. The SOD U/mg protein was used to express the results. Catalase (CAT) activity was determined by quantifying the reduction in H_2O_2 concentration at 240 nm[26]. The findings were reported as U/mg protein. Glutathione-S-transferase (GST) activity was based on the absorbance determination at 340 nm of 1-chloro-2, 4-dinitrobenzene and GSH conjugation[27]. Results were expressed as GST U/g protein. The thiobarbituric acid assay of Warren was used to determine the amount of sialic acid (SA)[28]. The findings were presented as µg SA/g of protein. The Lowry method was used to calculate total protein level[29], and the total protein content was used for determining the values of other parameters per protein.

Determination of tissue factor activity

Tissue factor (TF) activities of pancreas samples were evaluated according to Quick's one-stage method[30]. The presence of a prolonged clotting time indicates a reduction in TF activity.

Histological examination

The pancreas tissue fixed in 10% formalin was embedded in paraffin for the light microscopic examination. For general morphology, the tissue sections were obtained as thick as 5 µM and stained with hematoxylin-and-eosin (H & E). Then they were randomly selected and photographed with an Olympus DP 72 digital camera linked to the Olympus BX51 microscope (Olympus, Tokyo, Japan).

Statistical analyses

Statistical analyses were performed using GraphPad Prism 9.0.1 (GraphPad Software, San Diego, CA, USA). For every group, the data were presented as mean ± standard error of the mean (SEM). Statistical analyses used are one-way ANOVA and Tukey tests. *p*-values below 0.05 are regarded as significant.

Results

Evaluation of the body weight and blood glucose level

There were no significant alterations in body weight following one week of administering chard and VPA separately, or in combination (Table 1).

Blood glucose values at day 8 were significantly different between groups (P_{ANOVA}<0.0001, Table 2). In the VPA group, increased levels of blood glucose were observed when they were compared with the C group (P<0.001) and the C+Chard group (P<0.001). Administering chard to both the control and VPA groups led to a notable decrease in the levels of blood glucose compared to those in the C group and VPA group (P<0.001).

Evaluation of oxidant and antioxidant status of pancreatic tissue

The differences of pancreatic GSH and LPO levels were significant between groups (P_{ANOVA}<0.0001, Fig. 1). VPA significantly decreased GSH levels (P<0.05) and significantly increased the levels of MDA (P<0.05) when compared with control group. In the C+Chard and VPA+Chard groups, chard administration reversed the effects of VPA resulting in an increase GSH and a decrease MDA compared to C and VPA groups (P<0.001).

No significant differences were observed in SOD, CAT and GST activities between the VPA group and the C group (P> 0.05) (Fig. 2). A significant rise in SOD activity and a significant decline in CAT and GST activities were detected in the C+Chard group compared to the C group (P<0.05, P<0.05, and P<0.01, respectively). The CAT activity in the

Table 1 — Comparison of the body weights of the groups

Groups (n=8)	Body weight (g) *		$P_{t\text{-test}}$
	At the beginning	At the end	
C	142.23±6.31	141.41±6.26	>0.05
C+Chard	142.51±2.93	136.26±2.89	>0.05
VPA	142.80±4.31	141.83±3.10	>0.05
VPA+Chard	145.28±6.01	140.17±5.86	>0.05
P_{ANOVA}	0.7703	0.5328	

n: number of animals. *Values are mean ± SEM. SEM: Standard error of the mean. C: Control group, C+Chard: Chard given control group, VPA: Valproic acid given group, VPA+Chard: Valproic acid and chard given group

Table 2 — Comparison of the blood glucose levels of the groups at day 8

Groups (n=8)	Blood glucose level (mg/dL) *
C	77.15±0.06
C+Chard	73.28±0.90[a]
VPA	83.23±0.77[a, b]
VPA+Chard	74.83 ±1.14[c]
P_{ANOVA}	<0.0001

n: number of animals. *Values are mean ± SEM. SEM: Standard error of the mean. C: Control group, C+Chard: Chard given control group, VPA: Valproic acid given group, VPA+Chard: Valproic acid and chard given group.[a]P<0.001 *vs*. control, [b]P<0.001 *vs*. C+Chard, [c]P<0.001 *vs*. VPA

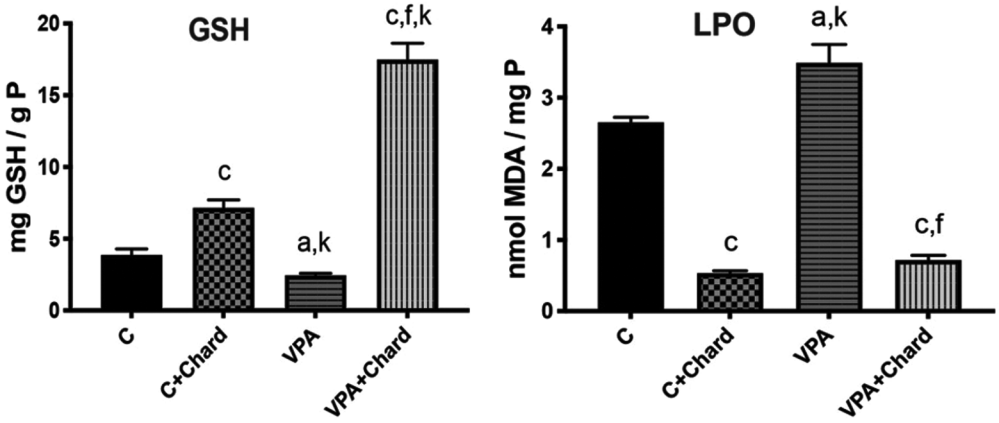

Fig. 1 — Pancreatic tissue GSH and LPO levels of all groups. The values are given as mean ±SEM, SEM: Standard error of the mean. C: Control group, C+Chard: Chard given control group, VPA: Valproic acid given group, VPA+Chard: Valproic acid and chard given group, P: Protein, GSH: Glutathione, LPO: Lipid peroxidation, MDA: Malondialdehyde, $^{a}P<0.05$, $^{c}P<0.001$ *vs* control, $^{f}P<0.001$ *vs* VPA group, $^{k}P<0.001$ *vs* C+Chard group, $P_{ANOVA}<0.001$; significant differences between groups

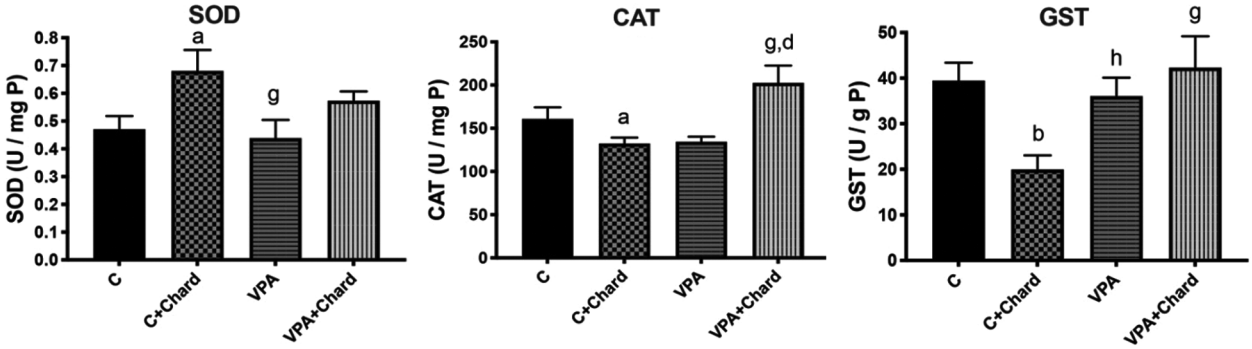

Fig. 2 — Pancreatic tissue SOD, CAT, and GST activities of all groups. The values are given as mean ±SEM, SEM: Standard error of the mean. C: Control group, C+Chard: Chard given control group, VPA: Valproic acid given group, VPA+Chard: Valproic acid and chard given group, P: Protein, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-S-transferase. $^{a}P<0.05$, $^{b}P<0.01$ *vs* control, $^{d}P<0.05$ *vs* VPA group, $^{g}P<0.05$, $^{h}P<0.01$ *vs* C+Chard group

VPA+Chard group was significantly higher than those of the VPA ($P<0.05$) and C+Chard groups ($P<0.05$). The VPA and VPA+Chard groups exhibited significantly greater GST activity ($P<0.01$ and $P<0.05$, respectively), compared to the C+Chard group.

The VPA administration did not alter levels of pancreatic SA compared to the C group. SA levels were significantly higher in both C+Chard and VPA+Chard groups compared to the respective groups (C and VPA groups) ($P<0.001$). The SA level in the VPA+Chard group was also higher than the C+Chard group ($P<0.001$). TF activity in the VPA group was shown to be considerably higher than the C group ($P<0.001$). Chard administration caused a significant increase in TF activities of the C+Chard group and VPA+Chard group compared with the respective groups (C and VPA groups) ($P<0.001$). The TF activity in the VPA+Chard group was also higher than the C group (Fig. 3).

Histological examination of pancreatic tissue

The microscopic examination of pancreas tissue showed a regular alignment with acinar cells and Langerhans islets (Fig. 4 A-D) for the control and chard groups. However, in the VPA group, acinar cell and Langerhans islets degeneration, eosinophilic fibrillar cytoplasm, and cytoplasmic vacuolization were present (Fig. 4C). The regeneration of Langerhans islets and acinar cells was seen in the VPA+Chard group (Fig. 4D).

Discussion

The therapeutic use of VPA is increasing with its use in chronic and prophylactic treatment settings. Therefore, the problem of adverse side effects is also becoming more important. The National Institutes of Health cautions patients who use VPA about the potential risks of liver and pancreas damage associated with the drug's usage[31]. The mechanism by

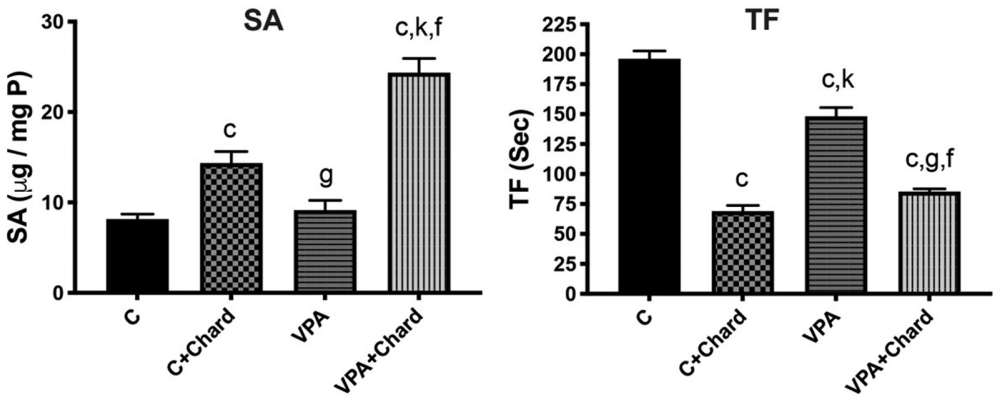

Fig. 3—Pancreatic tissue TF activity and SA levels of all groups. The values are given as mean ±SEM, SEM: Standard error of the mean. C: Control group, C+Chard: Chard given control group, VPA: Valproic acid given group, VPA+Chard: Valproic acid and chard given group, P: Protein, TF: Tissue factor, SA: Sialic acid, $^{c}P<0.001$ *vs* control, $^{f}P<0.001$ *vs* VPA group, $^{g}P<0.05$, $^{k}P<0.001$ *vs* C+Chard group

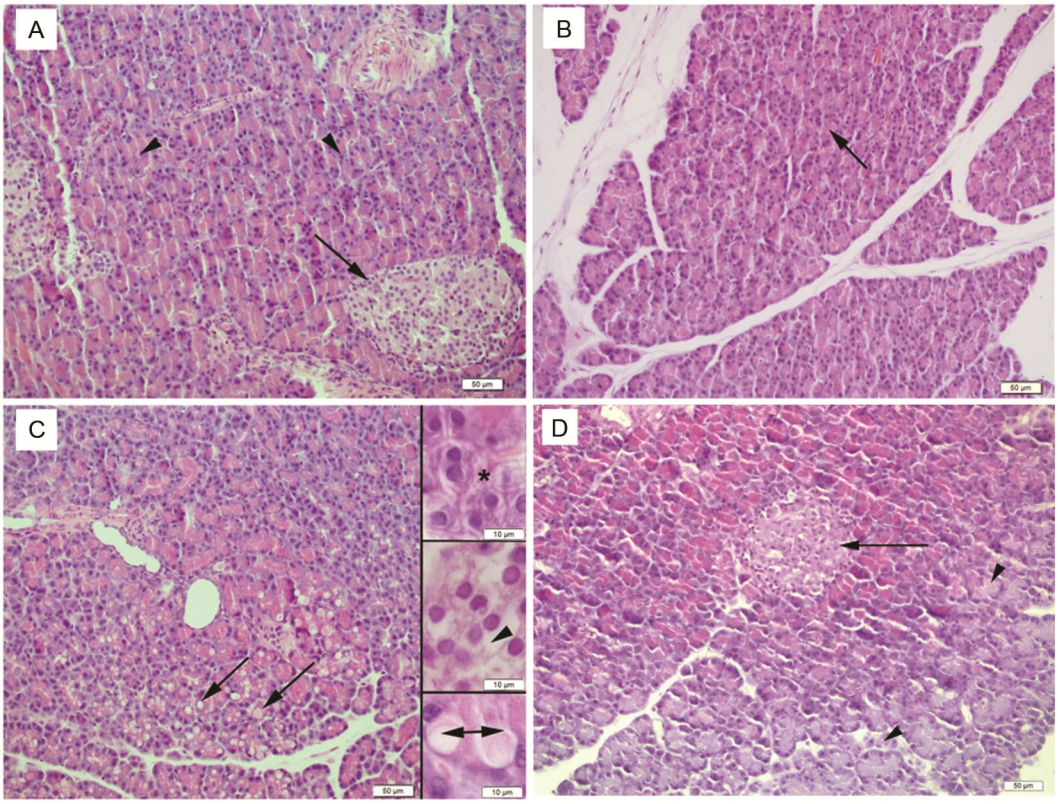

Fig. 4 —Representative light photomicrographs of pancreas tissue sections in the experimental groups. (A) Control group demonstrates a regular alignment with acinar cells (arrowheads) and Langerhans islets (arrow); (B) C+Chard group shows similar morphology with the control group, acinar cells (arrow); (C) VPA group has acinar cell degeneration (arrows) with cytoplasmic vacuolization (two-headed arrow-inset), eosinophilic fibrillar cytoplasm (asterisk) and degeneration of Langerhans islets (arrow head-inset); and (D) VPA+Chard group shows regeneration of acinar (arrowheads) and Langerhans islets (arrow) morphology. H & E staining. Original magnification: X200 and X1000 (inset)

which VPA causes pancreatic injury remains unclear. Since the proper functioning of the pancreas is vital for maintaining normal blood glucose levels, oxidative stress may play role(s) in the pancreas damage caused by VPA and antioxidants may potentially provide protection. Chard is explored in this study because it has antioxidant activity due to its high phenolics, flavonoids, and proline content[14]. In this study, we aim to investigate the possible protective effects of chard against pancreatic complications caused by VPA.

Acute pancreatitis has been linked to VPA as side effect in some peer-reviewed publications[32-35], but the precise underlying mechanisms of VPA-induced pancreatitis remain poorly understood. However, it is thought to be caused by several reasons, including the narrowing of pancreatic ducts, toxic effects on pancreatic cells, disruptions in metabolic processes, the accumulation of hazardous metabolites or intermediates, and possibly hypersensitive responses[32]. It has been suggested that the presence of free radical scavengers like glutathione peroxidase, CAT, and SOD are reduced with the use of VPA, which is associated with acute pancreatitis. This depletion of scavengers could potentially trigger an over-production of free radicals, subsequently causing increased lipid peroxidation and permeability of endothelial cells, eventually resulting in tissue impairment[33].

Oxidative stress could be implicated in the progression of insulin resistance and the impairment of β cell function[36]. Furthermore, there is a hypothesis that the decline in carnitine levels caused by VPA administration significantly contributes to the observed pancreatic damage. Another theory is proposed regarding VPA-induced pancreatitis involvement that VPA and its metabolites could potentially impair the mitochondrial β-oxidation of fatty acids by directly inhibiting enzymes involved in fatty acid oxidation[33]. Based on previous studies, pancreatitis resulting from valproate use has been described as an idiosyncratic adverse effect as well as a dose-dependent reaction; however, there is currently no confirmed link between the dosage or serum VPA level and its occurrence[34].

Pancreatitis has been documented to manifest as an idiosyncratic response, occurring anywhere from 1 week to 8 years after exposure to VPA in humans[35]. Our previous study demonstrated that biochemical and morphological alterations are detected in the pancreas following the administration of 500 mg VPA/kg dose for 7 days[37]. Based on this experience, the dosage and treatment duration of VPA in rats (500 mg/kg/day for 7 days) were selected/utilized in the present study. In the literature, there is an ongoing discussion regarding whether VPA has an impact on insulin synthesis and secretion, body weight, and insulin signaling. In patients receiving VPA, plasma glucose levels increase due to insulin resistance. However, in some cases of insulin resistance, patients may maintain normal glucose levels due to compensatory hyperinsulinemia. These compensatory mechanisms lose their effectiveness when the pancreatic β-cell function deteriorates[38].

Recent studies indicate that VPA influences the HOMA-IR (homeostatic assessment-insulin resistance) index, leading to lower fasting blood glucose levels due to hyperinsulinemia. This suggests the potential use of VPA in the treatment of diabetes mellitus[39]. However, the antidiabetic property of VPA still needs to be elucidated. In the study of Akindele *et al*., although not statistically significant, except on day 21 at the dose of 100 mg/kg, on days 7 and 21, the blood glucose levels in rats with normoglycemia increased in response to VPA at doses of 100 and 300 mg/kg[40]. In this study, VPA administration increased blood glucose levels but it was within the normal range. Chard administration resulted in a reduction in glucose levels observed in both the control and VPA groups. There have been reports indicating that chard can lower blood glucose levels[11,12]. The potential blood glucose-lowering effect of chard could be linked to the abundance of saponins and flavonoids that inhibit gluconeogenesis, glycogenolysis, and activities of α-amylase and α-glucosidase[41]. All the groups did not show statistically significant changes in body weight in our present study. Similarly, Iamsaard *et al*. found that intraperitoneal treatment with 500 mg/kg VPA administration did not change the body weight of male Wistar rats at the end of the day 8[42]. However, in a study conducted by Aktas *et al*., when male Sprague-Dawley rats were given 500 mg VPA/kg, their body weight significantly decreased by the end of the 14th day[43]. Abdelkader *et al*. stated that after the treatment with 250 mg VPA/kg/day for 14 days, male Sprague-Dawley rats' body weight significantly higher than in the control group[44]. The cause of weight gain induced by VPA is likely complex and cannot be attributed to a single mechanism. Elevated insulin levels and insulin resistance may lead to weight gain by stimulating appetite. The increase in appetite is a result of the disruption in the hypothalamic system caused by an elevation in GABA (gamma-aminobutyric acid) transmission[38].

In our study, an increased LPO level and TF activity and a decreased GSH level were found in the VPA group compared with the C group, which was consistent with previous VPA-induced pancreatic toxicity studies[37,45]. Increased LPO levels and reduction in GSH levels in the pancreas are

characteristics of pancreatic injury[46]. TF is a transmembrane protein of 45 kD and is constitutively expressed in a variety of cells in the body. It has a significant part in the activation of coagulation and inflammation[47]. The shortened clotting time is indicative of prolonged TF activity. The increased TF activity in tissue samples contributes to cellular damage[48]. Oxidative stress has been shown to convert inactive TF to active TF[49].

Chard extracts are potent free radical scavengers, metal chelators, and inhibitors of lipid peroxidation[12,14]. Several prior studies verified the protective effects of chard through altering the activities of antioxidant enzyme and non-enzymatic antioxidant levels in different tissues such as the brain, cardiac, femur, kidney, and liver[21,41,50]. Although the hypoglycemic agent potential of chard extract in the pancreas has been previously studied[11], its antioxidant properties have not been evaluated. In our study, the administration of chard led to elevated GSH levels and reduced LPO levels in the VPA-treated group. These results might be related to chard's high antioxidant activity, which considerably decreased the oxidative stress induced by VPA. We hypothesize that the presence of phenolic chemicals may be responsible for the antioxidant action. The increase in pancreatic TF activity of chard given control and VPA groups, might suggest hypercoagulable conditions. Chard is high in vitamin K, which is essential for the synthesis of proteins belonging to the gamma carboxyglutamate-protein family comprising blood coagulation factors[13].

Biomolecular studies into the impact of VPA on pancreatic tissues have been attributed to the toxic effects to increased levels of free oxygen radicals in pancreatic tissue, associated with deficiencies in SOD, CAT, and GPx enzymes. Additionally, VPA is known to inhibit histone deacetylase, leading to an imbalance in pancreatic recovery[51]. The reduction in activity of GST and level of GSH has been associated with the increase of oxidative stress and the decline of antioxidant status induced by VPA[52]. In our study, chard extract decreased CAT and GST activity while increasing SOD activity in the chard given control group. The reason forthe decreased CAT and GST activity can be attributed to the polyphenols and flavonoids of chard that have the potential to inhibit the GST and CAT activities[14,53,54]. The ingredient of polyphenols in chard have been summarized in publications[14,55]. Flavonoids, upon binding to sites distinct from the active site of catalase, induce conformational modifications that lead to the inhibition of enzyme activity[53]. In a study by Tunali *et al*., a similar decrease in the activity of GPx, glutathione reductase, GST, and SOD was observed due to chard administration inthe control group, although without achieving statistical significance[21]. In our study, it was showed that administering chard extracts to the VPA group resulted in an increase in CAT activity. The increase in CAT activity can be attributed to the chard's antioxidant properties.

SA is important for a number of physiological and pathological functions, such as cell-to-cell communication, viral and bacterial infections, and interactions between proteins and carbohydrates. In addition to the masking of recognition sites, SA also functions as a biological target that receptor proteins recognize and interact with as ligands[56]. In certain diseases, such as cancer, diabetes, alcoholism, renal illnesses, and inflammatory processes, plasma total SA levels have been observed to increase[57]. In our study, VPA did not induce any alteration in the quantity of SA. However, levels of pancreatic SA increased both in the control and VPA groups in response to chard administration. Similarly, chard has been found to increase SA levels in the gastric tissue[58]. The observed elevation in SA levels could potentially be attributed to an increase in sialic acid synthesis.

Prior studies have shown that the VPA administration leads to chronic atrophic pancreatitis, reduces acinar cell proliferation, retards the recovery of damaged pancreas, and induces degenerative alterations in Langerhans islets and acinar cells[45, 59]. In our present study, the cytoplasm of the acinar cell was eosinophilic fibrillar and vacuolated in the VPA group. Additionally, the degeneration of Langerhans islets was detected. These changes are consistent with the previously mentioned studies and supports the detrimental effects of VPA on the pancreas. The observed cytoplasmic patterns could contribute to digestion physiology[37]. The degenerated pancreatic islets in the VPA group are attributed to VPA-induced oxidative stress damage. Although the exact mechanism is unknown, an excessive presence of free radicals resulting from the depletion of radical scavenger due to VPA administration could cause increased permeability in vessel walls and lipid peroxidation, ultimately harming tissues[60]. The increase, although within the normal limits, in blood

glucose values in the VPA group also supports the disruption of the endocrine function of pancreatic islets. In the VPA+Chard group, treatment with chard markedly improved all the histological changes caused by VPA. In this study, the preventive effects of chard is demonstrated by the regeneration of Langerhans islets and acinar cells, which its antioxidant properties can explain.

Like any other translational research studies, our study also has certain limitations. The first limitation of this study was the lack of testing for serum lipase and amylase activity, which were employed as indicators to demonstrate pancreatic damage. The second limitation was the absence of evaluation of pancreatic edema, which is used to estimate the degree of recovery following pancreatic damage.

Conclusion

Chard may have a protective effect against pancreatic complications caused by VPA, possibly through mechanisms including the inhibition of lipid peroxidation, and augmentation of antioxidants. Chard's ability to increase sialic acid levels and reduce blood glucose levels may also have significant therapeutic implications.

Acknowledgement

Prof. Dr. Kerim Alpınar identified the plant (Voucher Specimen Number: 67901) at Faculty of Pharmacy of Istanbul University, and the authors are grateful to him.

Conflict of interest

All authors declare no conflict of interest.

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