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ARAŞTIRMA / RESEARCH

Timokinon'un sıçanların pankreas dokusunda valproik asidin neden olduğu hasarı iyileştirmeye etkisi

Effect of thymoquinone on ameliorating valproic acid-induced damage in pancreatic tissue of rats

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Abstract

Purpose: The aim of this study was to evaluate the potential effect of thymoquinone (TQ) in preventing or treating valproic acid (VPA)-induced pancreatic injury, gene expressions and histopathological changes in pancreatic tissue of rats..

Materials and Methods: 21 male Spraque-Dawley rats were divided into 3 groups (n = 7): Control, VPA, VPA + TQ. Rats were treated orally with VPA (500 mg/kg/day) and TQ (50 mg/kg/day) for 14 days. On the 15th day of treatment, pancreatic tissue was resected for the analysis of the expression levels of histone deacetylase (HDAC1 and HDAC2) genes. Structural changes in pancreatic tissues were examined.

Results: It was observed that VPA + TQ group had significantly higher HDAC1 and HDAC2 genes expression compared to the VPA group. Furthermore, the findings show that TQ therapy can protect pancreatic tissue against the negative effects of VPA on HDAC1 and HDAC2 gene expression. It also decreased the levels of NADPH oxidase-4 (NOX-4) and caspase-3 (CAS-3). Oxidative stress decreased, antioxidant activity increased and histopathological changes decreased in the VPA + TQ group compared to the VPA group. Pancreatic damage caused by VPA was attenuated due to TQ's antioxidative and antiapoptotic effects.

Conclusion: TQ may be useful for reducing the severity of VPA-induced pancreatic damage.

Keywords: thymoquinone, valproic acid, apoptosis, toxicity, HDAC1/2

Öz

Amaç: Bu çalışmanın amacı timokinonun (TQ) valproik asit (VPA) kaynaklı pankreas hasarını önleme veya tedavi etmedeki potansiyel etkisinin, sıçanların pankreas dokusundaki gen ekspresyonlarının ve histopatolojik değişikliklerin değerlendirilmesidir.

Gereç ve Yöntem: 21 erkek Spraque-Dawley sıçanı 3 gruba ayrıldı (n = 7): Kontrol, VPA, VPA + TQ. Sıçanlar, 14 gün boyunca oral yoldan VPA (500 mg/kg/gün) ve TQ (50 mg/kg/gün) ile muamele edildi. Muamelenin 15. gününde, histon deasetilaz (HDAC1 ve HDAC2) genlerinin ekspresyon seviyelerinin analizi için pankreas dokusu çıkarıldı. Pankreas dokularındaki yapısal değişiklikler incelendi.

Bulgular: VPA + TQ grubunun, VPA grubuna göre anlamlı derecede daha yüksek HDAC1 ve HDAC2 gen ekspresyonuna sahip olduğu görüldü. Ayrıca bulgularımız, TQ tedavisinin pankreas dokusunu VPA'nın HDAC1 ve HDAC2 gen ekspresyonu üzerindeki olumsuz etkilerine karşı koruyabildiğini göstermektedir. Ayrıca TQ NADPH oksidaz-4 (NOX-4) ve kaspaz-3 (CAS-3) düzeylerini de düşürdü. VPA+TQ grubunda VPA grubuna göre oksidatif stres azaldı, antioksidan aktivite arttı ve histopatolojik değişiklikler azaldı. VPA'nın neden olduğu pankreas hasarı, TQ'nun antioksidatif ve antiapoptotik etkileri nedeniyle hafifletildi.

Sonuç: TQ, VPA'nın neden olduğu pankreas hasarının şiddetini azaltmada faydalı olabilir.

Anahtar kelimeler: timokinon, valproik asit, apoptoz, toksisite, HDAC1/2

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INTRODUCTION

Valproic acid (VPA), a carboxylic acid analog of valeric acid and a component of the *Valeriana officinalis* plant, is widely used in the treatment of epilepsy[1]. VPA is a fatty acid with anticonvulsant properties for the treatment of various epilepsy and seizure types such as myoclonic, generalized and partial seizures[2]. However, VPA's therapeutic mechanism of action is still not fully understood. Although VPA is known to be a relatively safe drug in high doses and long-term use, it can cause life-threatening side effects such as hemorrhagic pancreatitis, coagulopathies, bone marrow suppression, hepatotoxicity, and encephalopathy[3]. Until now, many studies have reported that Fanconi syndrome is associated with VPA therapy[4].

Acetylation and deacetylation of chromatin-associated histone proteins play an important role in the regulation of transcription and other essential cellular activities[5]. Histone deacetylases (HDACs) are enzymes that regulate many cellular processes by catalyzing the removal of acetyl groups on histone and non-histone proteins from lysine residues[6]. The HDAC family is divided into four classes (I, IIa / IIb, III and IV) but VPA only targets Class I (HDAC1-3 and 8) and Class IIa (HDAC4-5, 7 and 9) HDACs[7]. It was shown in studies that HDAC1 / 2 play critical role in DNA damage signaling[8] in T cell development and preservation of genomic stability[9] and increased tumor growth *in vivo*[10].

Today, HDAC inhibitors are known as potential anticancer agents and show promise in the treatment of many diseases[11]. VPA which acts as a histone deacetylase (HDAC) inhibitor has been used to treat neurodegenerative, HIV and cancer diseases in recent studies[12]. VPA affects many important events in gene transcription including cell cycle control, DNA repair, differentiation and apoptosis by suppressing HDACs[13]. VPA specifically targets HDAC1 and HDAC2 which are localized in the nucleus. VPA can stimulate less mature cell formation by targeting HDAC2.

The pancreas is a glandular organ responsible for various homeostatic functions in our body. Hormone is produced from the endocrine islet cells of the pancreas to regulate the blood sugar level, and enzymes are secreted from the exocrine acinar cells to facilitate digestion[14].

Acute pancreatitis, an inflammatory disease of the pancreas, is the main reason for hospitalizations for gastrointestinal disorders in many countries[15]. Acute pancreatitis occurs when protective mechanisms that prevent trypsinogen activation or decrease trypsin activity become ineffective. Activation of the enzyme in the pancreas leads to local inflammation and self-digestion of the gland[16]. Pancreaticobiliary anomalies, genetic predisposition, environmental exposure are among the causes of pancreatitis. Another cause of pancreatitis is VPA, an antiepileptic drug[15]. However, the mechanisms by which drugs cause pancreatitis are unknown.

HDACs mediate and provide pancreatic development. Nevertheless, VPA causes pancreatitis by inhibiting HDACs. While inhibiting HDACs, VPA triggers an imbalance in pancreatic healing and predisposes patients to pancreatitis[17]. It is reported that approximately 300,000 new cases occur each year in the United States of America[18]. In general, the mortality rate for acute pancreatitis is 10-15%, while the mortality rate for patients with severe acute pancreatitis is as high as 30-40%[19].

On the other hand, thymoquinone (TQ) is one of the main components of the essential oil of *Nigella sativa* seeds[20] and is used in the treatment of various diseases[21]. TQ is a molecule with many biological and medical activities with antitumor, anti-inflammatory, antioxidant[22,23], antimicrobial, anticancer[24] antiepileptic[25], anti-hypertensive[26] immunomodulatory and anti- inflammatory effects[20].

It has been shown in studies that TQ significantly slows down hepatocellular carcinoma cells depending on the concentration and is a promising anticancer compound for the treatment of hepatocellular carcinoma[27]. In another study, TQ is found to be inhibiting human umbilical vein endothelial cell migration, invasion, proliferation, and tube formation[28]. These results reveal that TQ inhibits tumor growth and angiogenesis, and can also be used as a potential drug for cancer treatment[29].

It has been detected that TQ is effective in the death of pancreatic cells induced by chemotheropathic compounds by reducing nuclear factor-B-dependent antiapoptotic genes, and the combination of antitumoral drugs and TQ increases growth inhibition[30]. TQ and *N. sativa* oil have been found to have a gastroprotective effect[31] and this effect is partially related to their radical scavenging effect and

the protection of the redox state in the gastric mucosa[32]. In addition, TQ has a protective effect on nephrotoxicity caused by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin[33].

Although there are many scientific studies on the fact that VPA causes acute pancreatitis, there is no study on the protective property of TQ against the side effects of VPA in the pancreas. In this study, it is aimed to show the efficacy of TQ in preventing or treating VPA-induced pancreatic damage by evaluating gene expressions and histopathological changes in the pancreatic tissue of rats.

MATERIALS AND METHODS

Chemicals

VPA, TQ (purity > 98%) and the other chemicals were obtained from Sigma Aldrich. Chemical Co. (St. Louis, MO).

Animal experiments

The experiments were carried out on 21 male Sprague-Dawley rats, weighing approximately 200-300 g and obtained from Fırat University Experimental Animal Research Center. Animals were maintained on a 12/12 hour light/dark cycle with free access to food and water at a controlled temperature of 21 °C. Approval (Protocol # 2017/134) for this study was obtained from the Ethics Committee of Fırat University Faculty of Medicine.

Experimental design

The rats were randomly divided into 3 groups of 7 animals each: the control group (saline solution), the VPA group (500 mg / kg), the VPA + TQ group (500 mg / kg VPA and 50 mg / kg TQ). Animals were treated with VPA (500 mg / kg) and TQ (50 mg / kg) doses were administered orally daily with gavage once a day for 14 days. The administered doses of VPA[34] and TQ[35] were taken from previous studies.

At the end of the 14th day, the rats were euthanized under anesthesia by intraperitoneal injection of ketamine and xylazine. Pancreatic tissues were excised and quickly washed with cold saline. Each pancreas was divided into two parts. A portion was maintained at -80 °C until analysis of gene expression. The other part was fixed in 10% neutral formalin for histopathological evaluation.

Real-time PCR analysis

Frozen pancreatic tissue (30 mg) was homogenized (Bioprep-24, Allsheng) for 1 minute in 500 µl of Tissue Lysis Buffer. ExiPrepTM Tissue Total RNA isolation kit (Bioneer, K-3325) was used to obtain total RNA. RNA concentration was determined at absorbance at 230-260 nm and 260/280 nm with a NanoDrop spectrophotometer (Denovix DS-11). AccuPower® RT PreMix (Bioneer, K-2041) was reverse transcribed into cDNA following the manufacturer's instructions. The mRNA expression levels of HDAC1 and HDAC2 genes were determined using the ExiCyclerTM96 Real Time Quantitative PCR system (Bioneer). The cDNA was used for PCR amplification under the following conditions: 5 minutes at 95 °C, followed by 45 cycles of 15 seconds at 95 °C, followed by 60 °C for 25 seconds. The primer sequences used for HDAC1 and HDAC2 (Bioneer, S-1001) were shown in Table 1. The expression levels of the genes were calculated by the $2^{-\Delta\Delta Ct}$ method.

Table 1. Nucleotid sequences of primers

Name	Sequence (5'-3')
HDAC1	Forward primer: 5'-GCGAGCAAGATGGCGCAGACT-3' Reverse primer: 5'-GTGAGGCTTCATTGGGTGCCCT-3'
HDAC2	Forward primer: 5'-CTCCGGGCTGTCCTTGCTGC-3' Reverse primer: 5'-GCCGCCTCCTTGACTGTACGC-3'

HDAC1: histone deacetylase 1; HDAC2: histone deacetylase 2

Histochemical analysis

Pancreatic tissues obtained from animals were taken into 10% neutral formalin solution and fixed. After the fixation was completed, the tissue samples were taken to the washing process. The washing process is respectively as: passing through alcohol batches, dehydration, making transparent with xylol and embedding in paraffin. 4-5 µm sections were taken from the obtained paraffin blocks. Samples from all groups were stained with Hematoxylin-Eosin for

histopathological evaluation. Histopathological findings were evaluated under the following headings: vacuolization (acinar and islet), deterioration in islet morphology, perivascular fibrosis, interlobular connective tissue increase. A modified semi-quantitative scoring system was used to evaluate the findings. (-): no finding, (+): low level of finding (++): moderate finding, (+++): intensive finding). The images of the samples were taken using a photomicroscope.

Immunohistochemical analysis

4-5 μm sections obtained from paraffin blocks were lysed. Sections were deparaffinized with heat and xylol. The sections were then passed through alcohol batches, washed with PBS. They were incubated with 3% hydrogen peroxide, non-immune block solution, primary antibody, secondary antibody, streptavidin peroxidase, respectively. Marking with DAB solution and core staining with hematoxylin were done. Immunoreactivity was scored as: (-), no staining; (+), weak staining; (++), moderate staining; (+++), intense staining.

Statistical analysis

In our study, SPSS software programme (version 20.0) was used for statistical analysis. Data were given as means ± SEM. Shapiro-Wilk test was used for the evaluation of the normality. For the comparisons of HDAC1 and HDAC2 gene expression levels between the groups, one-way ANOVA after post-hoc LSD was used. In histological and immunohistochemical studies, because of the measurement values did not show homogeneous distribution, Kruskal Wallis test from nonparametric tests was performed for the comparison of groups. A p-value less than 0.05 ($p \leq 0.05$) was accepted statistically significant.

RESULTS

Table 2 shows the effects of TQ treatment against the VPA administration on the mRNA expression of HDAC1 and HDAC2 genes level in all study groups and control. HDAC1 and HDAC2 genes expression was significantly reduced in VPA group compared to the control group ($p \leq 0.05$). The VPA + TQ group had a significantly lower expression of HDAC1 and HDAC2 genes level compared to the VPA group ($p \leq 0.01$) (Table 2).

Normal pancreatic histology was observed in the sections belonging to the control group (Figure 1 A, B). Widespread vacuolization, increased interlobular connective tissue and perivascular fibrosis were observed in both islets and acinar cells in the VPA groups. No abnormality was detected in the size and shape of the islet (Figure 1 C, D). In the TQ groups, it seemed that there was decrease in vacuolization in islets and acinar cells, perivascular fibrosis and interlobular connective tissue compared to the VPA group. It was observed that the use of TQ reduced the pathological changes in the VPA + TQ group. Islets were observed in normal morphology in size and shape (Figure 1 E, F). All findings are summarized in Table 3.

Table 2. Effects of VPA and TQ on the expression of HDAC1 and HDAC2 genes in rat pancreatic tissue

Groups	Expression value (Ct value; Mean ± SEM)	
	HDAC1	HDAC2
Control	$30.56 \pm 0.47^{b,c}$	$35.43 \pm 0.20^{b,c}$
VPA	$25.58 \pm 0.58^{a,c}$	$33.58 \pm 0.33^{a,c}$
VPA + TQ	$23.42 \pm 0.18^{a,b}$	$31.55 \pm 1.02^{a,b}$

Each group represents the mean ± SEM for seven rats. a: Significant from control; b: Significant from VPA; c: Significant from VPA + TQ. VPA, valproic acid; TQ, thymoquinone; HDAC1: histone deacetylase 1; HDAC2: histone deacetylase 2

Table 3. Histopathological scoring of pancreas sections of experimental groups

Parameters/scores	Control	VPA	VPA + TQ
Vacuolization	–	$++^{a}$	$+^{b}$
Interlobular connective tissue increase	–	$++^{a}$	$+^{b}$
Perivascular fibrosis	–	$++^{a}$	$+^{b}$
Disruption in islet morphology	–	–	–

Scoring as described in the Methods section. n = 7. VPA, valproic acid; TQ, thymoquinone; *a*: VPA increased pancreatic damage, $p < 0.05$ vs. control group. *b*: TQ reduced pancreatic damage, $p < 0.05$ vs. VPA

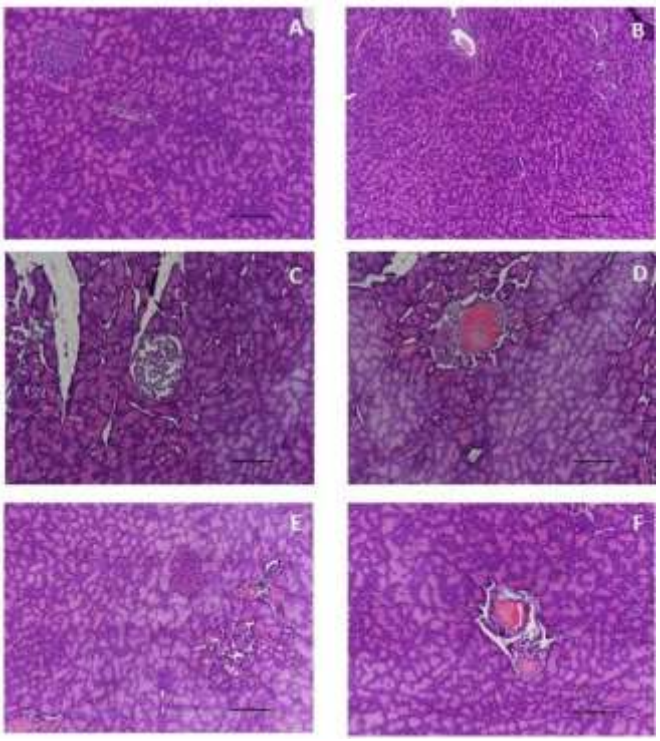

Figure 1. Rat pancreatic tissue section. A, B) Normal histology of the pancreatic tissue in the control group. C, D) Diffuse vacuolization, increase in interlobular connective tissue and perivascular fibrosis in both islets and acinar cells in VPA groups. E, F) It was observed that the use of TQ reduced the pathological changes in the VPA + TQ group. H&E, scale bar 50 μm, x400.

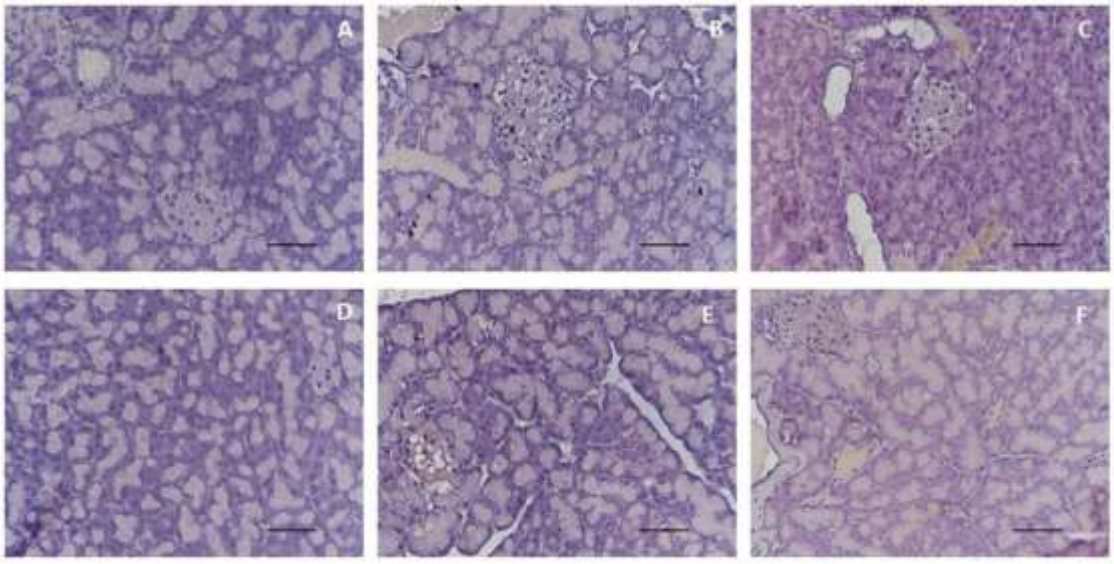

Figure 2. CAS-3 immunostaining. A) Control group. B) VPA group. C) VPA + TQ group. NOX–4 immunostaining. D) Control group. E) VPA group. F) VPA + TQ group. Scale bar 50 μm, x400. VPA, valproic acid; TQ, thymoquinone.

Table 4. Immunoreactivity scores for CAS-3 and NOX-4

	Control	VPA	VPA + TQ
CAS-3	(-)	(+++)[a]	(++)[b]
NOX-4	(-)	(++)[a]	(+)[b]

n = 7. VPA, valproic acid; TQ, thymoquinone; CAS-3, caspase 3; NOX-4, NADPH oxidase-4. a: VPA increased pancreatic damage, $p < 0.05$ vs. control group. b: TQ reduced pancreatic damage, $p < 0.05$ vs. VPA

The immunohistochemical findings are shown in Table 4 and Figure 2. CAS-3 immunoreactivity was detected in the pancreatic tissues of the control group (Figure 2A). CAS-3 immunoreactivity was detected in the VPA group (Figure 2B), while moderate CAS-3 immunoreactivity was detected in the TQ group (Figure 2C). NOX-4 immunoreactivity was not detected in the pancreatic tissues of the control group (Figure 2D). Moderate NOX-4 immunoreactivity was detected in the VPA group (Figure 2E), while weak NOX-4 immunoreactivity was detected in the TQ group (Figure 2F).

DISCUSSION

As an antiepileptic drug, VPA is generally used in epilepsy and psychiatric disorders[36]. Nonetheless, liver dysfunction, pancreatitis, anorexia, hyperammoniemia, and thrombocytopenia have been reported among the side effects of VPA therapy[37]. In addition, it may cause hepatotoxicity as a result of increased oxidative stress[38]. Several studies have shown that HDACs which regulate the signals that enable pancreatic cells to differentiate are upregulated during embryonic development in the pancreas and during recovery and regeneration after pancreatic injury[39]. It has been also demonstrated that VPA reduces pancreatic regeneration by inhibiting HDAC activity and triggers an imbalance in pancreatic healing, causing pancreatitis and delayed pancreatic recovery when giving VPA to mice after they develop pancreatitis[17].

In normal circumstances, HDAC activity has been shown to increase after pancreatic injury. HDACs are important epigenetic keys to the correct completion of regenerative programs. The mechanisms underlying VPA causing pancreatitis can be summarized as reduction of proliferation of acinar cells, persistence of ADMs (acinar-to-ductal metaplasia) and prevention of re-differentiation in acinar[17]. Although the success of HDACi against solid tumors is limited, it has shown efficacy in clinical studies on hematological cancers[40]. Some studies have shown that mice receiving VPA after pancreatitis induction have 40% to 50% reduced acinar content[17]. During pancreatic healing of HDACs, especially HDAC1 is upregulated. HDAC1 and HDAC2 which are class I HDACs are localized in the nucleus[41]. VPA suppresses gene transcription primarily by inhibiting HDAC1 and HDAC2[42].

In this study, VPA treatment reduced HDAC1 and HDAC2 gene expressions. Previous studies revealed that in an experimental pancreatic injury model, VPA was found to delay healing and reduce acinar cell proliferation in the pancreas. VPA was found to shift the balance towards pancreatic injury and pancreatitis by HDAC inhibition. Additionally, the increased production and accumulation of the VPA metabolite may be responsible for pancreatic damage in susceptible individuals[43]. In a study conducted on pregnant rats, it was observed that the pancreas of rats born from pregnant rats had a low immune reaction to insulin as a result of the effect of VPA exposure on HDAC activity which controls the expression of genes involved in the development of beta cells in the pancreas[44]. In another study, the combination of 5-fluorouracil and VPA significantly reduced cell viability by 30%[45]. According to the studies, VPA impairs endocrine pancreatic development during pancreatic development in zebrafish embryos and impairs pancreatic development by delaying exocrine pancreas formation, thus affecting the liver[46]. Some studies revealed that VPA treatment inhibited the HDAC1/2 activity, leading to autophagy and apoptosis in gastric cancer cells[47].

In this study, a significant decrease was found in HDAC2 protein expression and especially HDAC1 expression after VPA treatment. Our findings are supported by the results of a previous study[47]. Many HDAC inhibitors have limited therapeutic use either because they exhibit significant toxicity or because of poor bioavailability[48]. Previous studies have shown that TQ has been found to be highly effective in inhibiting different cancer stages such as proliferation, migration and invasion. TQ exhibits antineoplastic activity in many types of cancer, including breast, sarcoma, prostate, colorectal, and

leukemia[49]. TQ regulates the levels of pro- and anti-apoptotic genes and induces apoptosis. TQ, as the most abundant component of *Nigella sativa* seed's essential oil, has antioxidant and anti-inflammatory activity. Therefore, in this study, the VPA + TQ group had a significantly lower HDAC1 and HDAC2 genes level compared to the VPA group.

According to a studies, TQ inhibits the proliferation of multiple myeloma cells through the modulation of various proliferation, survival and angiogenesis markers[50]. It has been shown that patients with chronic pancreatitis have an increased risk of extrapancreatic cancers[51]. A study has found out that TQ, a HDACi, cures cancer associated with inflammation, inhibits proliferation in pancreatic ductal adenocarcinoma cells and induces apoptosis[52]. The studies on TQ have shown that it inhibits the growth and progression of cancer through activation or inactivation of molecular cell signaling pathways in various cancer cells without any toxic effects on normal cells[53]. TQ targets multiple molecular pathways in cancer cells in different ways. TQ exerts anti-neoplastic action through various cellular mechanisms such as inhibition of cell proliferation, induction of apoptosis, and inhibition of the production of reactive oxygen species (ROS)[54].

According to the histological results of this study, treatment with VPA predisposes the pancreas to pancreatitis. As stated in the findings, diffuse vacuolization, increased interlobular connective tissue and perivascular fibrosis were observed in both islets and acinar cells in the VPA groups. In the TQ groups, vacuolization in islets and acinar cells, perivascular fibrosis and interlobular connective tissue were decreased compared to the VPA group. It was observed that the use of TQ reduced the pathological changes in the VPA + TQ group. The histopathological findings show that TQ protects the histological structure of the pancreas against damage caused by VPA.

Oxidative stress caused by VPA stimulates various signaling pathways including activation of CAS-3 which causes apoptosis[55]. In mammalian cells, CAS-3 acts as a marker for apoptosis[56]. VPA treatment increases the activity of CAS-3 in pancreatic tissues and thus induces apoptosis[57]. Increased CAS-3 immunoreactivity in the VPA-treated group indicates apoptosis of pancreatic tissues. Besides, the CAS-3 level in pancreatic tissues of the VPA + TQ-treated group was lower than that of the VPA group. In this study, it was found out that the CAS-3 level was significantly reduced in rats treated with TQ. According to the results, it has been reported that TQ can be useful in maintaining the apoptosis rate within normal limits[58]. The findings suggest that TQ can protect against pancreatic damage caused by VPA, through to its antioxidant effect.

NOX-4 is an important source of apoptosis and oxidative stress in pancreatic tissue and participates in pancreatic dysfunction[59]. Inhibition of NOX-4 could be an alternative strategy for the treatment of VPA-induced pancreatic apoptosis. Previous researches have shown that exposure to VPA can lead to an increase in ROS, causing DNA double-strand breaks that can lead to ROS-induced DNA damage and deleterious genetic changes[60] as well as teratogenicity[61]. In this study, it was observed that the NOX-4 level in the VPA group increased significantly compared to the VPA + TQ group. Therefore, it can be suggested that VPA-induced ROS causes oxidative stress in pancreatic tissues. The researchers of this study confirmed that TQ reduces VPA-induced oxidative stress by inhibiting NOX-4 level, consistent with a previous report[62]. Treatment with TQ also reduced ROS formation and reduced VPA-induced pancreatic apoptosis.

TQ attenuated the VPA-induced decline in cell viability. It has been found out that TQ helps preventing pancreatic damage by inhibiting adverse effects on VPA-induced gene expressions, reducing oxidative stress and preventing histological changes. As a result, HDACs regulate pancreatic regeneration through re-differentiation of ADMs into acinar cells. VPA, on the other hand, prevents ADMs from differentiating into mature acinar cells and causes pancreatitis. Previous studies have shown that treatment with VPA increases the formation of ROS and causes a significant decrease in cellular viability[63]. In this study, it has been revealed that VPA reduces acinar cell proliferation of the pancreas. Accordingly, VPA shifts the balance towards pancreatic damage and pancreatitis through HDAC inhibition. It has been observed that the TQ used in this study helps to correct the damage caused by VPA by minimizing it.

The limited number of rats in the study due to the 3R rule, the longer use of VPA in humans, and the lack of knowledge of other side effects that may occur in the long term and the suitability of doses on experimental animals for clinical studies are among the limitations of this study. This study can guide clinical studies on this subject.

It has been revealed that TQ reduces apoptosis of pancreatic cells, suppresses oxidative stress, prevents histological changes, and consequently, protects rat pancreatic tissues from VPA-induced damage. Accordingly, TQ appears to be a potential aid in application with VPA to ameliorate pancreatic damage. Therefore, the results suggest that TQ may be beneficial in reducing VPA-induced pancreatic damage.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SA, SB, DTK, MS, MKÖ; Veri toplama: SA, SB, DTK, MS; Veri analizi ve yorumlama: SA, SB, MS; Yazı taslağı: SA, SB; İçeriğin eleştirel incelenmesi: SA, SB, DTK, MS, MKÖ; Son onay ve sorumluluk: SA, SB, DTK, MS, MKÖ; Teknik ve malzeme desteği: SA, SB, DTK, MS; Süpervizyon: SA, SB, DTK, MS, MKÖ; Fon sağlama (mevcut ise): yok.
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