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Tauroursodeoxycholic Acid (TUDCA) regulates inflammation and hypoxia in autonomic tissues of rats with seizures

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ABSTRACT

Inflammation and hypoxia have an effect on the molecular mechanism of cardiovascular and respiratory pathologies accompanying seizures. Against this, Tauroursodeoxycholic Acid (TUDCA) can regulate oxidative stress, inflammation and cellular survival by suppressing endoplasmic reticulum (ER) stress. We evaluated the expression changes of NF-κB p65, TNF-α, HIF1α and Kir6.2 proteins associated with seizures in brain stem, heart and lung tissues representing the autonomous network. Additionally, we examined the protective effects of TUDCA administration against damage caused by seizures in terms of immunohistochemistry and pathology.

4 groups of Wistar Albino male rats (250-300 g, n=32) were formed as control, pentylenetetrazole (PTZ), TUDCA and PTZ+TUDCA. The epilepsy kindling model was created by intraperitoneal (i.p.) injection of PTZ chemical (35 mg/kg, every 2 days) for one month. TUDCA (500 mg/kg; every 2 days) treatment was given intraperitoneally 30 minutes before seizures for 1 month. Brain stem, heart (atria, ventricle) and lung tissues of rats were isolated. NF-κB p65, TNF-α, HIF1α and Kir6.2 proteins in the obtained tissues were evaluated by immunohistochemical staining.

The immunoreactivity of the investigated proteins in the brainstem heart and lung tissues of rats with chronic PTZ administration was significantly increased. Recurrent seizures led to accumulation of inflammatory cells in tissues, hemorrhage, vasodilation, and apoptosis. Following TUDCA administration, expression of NF-κB p65, TNF-α and Kir6.2 was significantly reduced in all tissues (except the atrium of the heart) compared to control rats. HIF-1α levels were significantly suppressed in ventricular and lung tissues of epileptic rats given TUDCA. However, TUDCA pretreatment improved histopathological changes due to chronic seizures and partially reduced apoptosis.

We showed that epileptic seizures may cause tissue damage with the development of inflammatory and hypoxic conditions in the brainstem and organs that represent the autonomic network. TUDCA therapy could be an effective agent in the treatment of cardiac and respiratory problems associated with seizures.

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Introduction

About 2% of the world's population suffer from epilepsy, a common neurological disease. This disease progresses with seizures that occur spontaneously as a result of the deterioration of the balance between excitation and suppression in neurons (1,2). Epileptic networks are closely linked to the autonomic nervous system that regulates respiratory and cardiovascular functions. Seizures can trigger death by causing autonomic disorders (3,4). The emergence and recurrence of epileptic seizures is associated with the activation of inflammatory cells and molecules (5). In this context, inflammation and hypoxia may reflect a fundamental target in the pathogenesis of seizure-induced autonomic disorders.

Inflammation can induce seizure formation and cell damage by causing hyperexcitability (6). Nuclear factor kappa B (NF-κB), which belongs to the family of transcription factors, regulates the expression of various proinflammatory mediators such as cytokines and chemokines (7). NF-κB p65 molecule is the predominantly activated subtype from the family (8). Available findings highlight that NF-κB is upregulated in epilepsy by several mechanisms like neuronal loss and glial activation (9). However, the effect of the NF-κB p65 molecule on autonomic disorders accompanying seizures is unknown.

Tumor necrosis factor alpha (TNF-α), which can accompany the formation of the inflammatory response by inducing various cytokines and lipid mediators, is a precursor cytokine in inflammation (10). TNF-α can also in-

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crease glutamergic transmission by upregulating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (11). Herein, TNF-α, a pro-inflammatory protein, may act as a crucial response element of the inflammation mechanism in the formation of epileptic seizures.

Hypoxia is a prominent feature of the inflammatory microenvironment (12). The hypoxia-inducible factor 1 alpha (HIF1α) molecule is the main regulator providing cellular adaptation to a low oxygen environment (13). HIF1α stimulates the production of genes that control oxygen balance and metabolic activation at the transcriptional level (14). In this context, HIF1α may be associated with respiratory and cardiovascular pathologies accompanying seizures.

Kir6.2 from the inwardly rectifying K^+ channel (Kir) family, may provide neuronal protection by regulating the membrane potential in hypoxic conditions (15). Kir6 channels function as molecular rheostats that regulate membrane potential in accordance with cellular energy needs (16). Suppression of energy expenditure via Kir6 channels during seizures may be a protection mechanism against cellular damage (17). Kir6.2 channels may be effective in controlling cellular damage in brain stem, heart and lung tissues affected by seizure-related hypoxia.

The production of tauroursodeoxycholic acid (TUDCA), a hydrophilic bile acid, occurs naturally in the liver. Besides reducing endoplasmic reticulum stress (ER) and stabilizing unfolded protein stress, TUDCA compound plays a chemical chaperone role. This compound shows cytoprotective effects by suppressing oxidative stress, inflammation and apoptosis in cell culture and in vivo models (18,19). In this direction, TUDCA may have a positive effect against apoptotic cellular death in the pathogenesis of epilepsy.

In the pathogenesis of cardiovascular and respiratory disorders that accompany seizures; inflammation and hypoxia-related pathways may be involved. Imaging data of epilepsy patients with generalized tonic-clonic seizures reveal volume losses in brainstem regions that regulate cardiac and respiratory activity, related to the severity of hypoxia (20). However, studies showed that the pro-inflammatory cytokines such as TNF-α expressions is increased due to ER stress in the brain tissues of genetic epilepsy model mice. These findings may indicate that ER stress caused by recurrent seizures may lead to autonomic disorders. In our study, we evaluated the expression of NF-κB p65, TNF-α, HIF1α and Kir6.2 in brain stem, heart and lung samples of rats with epilepsy model. Also, complex role of seizure markers, including cytokines, in autonomic function control was investigated for the first time. We investigated the protective representation of pretreatment against seizure damage with an ER stress inhibitor TUDCA at the histopathological and immunohistochemical levels. According to our results, TUDCA therapy could be used as an effective agent in the treatment of cardiac and respiratory problems associated with seizures.

Materials and Methods

Ethical approval with protocol number 21/178 was obtained from Erciyes University Animal Experiments Local Ethics Committee (HAYDEK). 2-3 months / 8-12 weeks old, weighing 250-300 g, 32 adult wistar albino male rats, were taken from Erciyes University Experimental and Clinical Research Center (DEKAM). Rats were housed in a controlled environment (24 ± 2°C and 60% humidity) under a 12-hour light-dark cycle, and were provided with free access to tap water and standard food throughout the experiment. In the controlled environment (24 ± 2°C and 60% humidity) under a 12-hour light-dark cycle rats were housed. Rats were supplied with free access to tap water and standard food throughout the experiment.

Recommendations from the Guidelines for the Care and Use of Laboratory Animals, adopted by the National Institutes of Health (USA) and the Declaration of Helsinki were fully followed, and procedure have been applied. To minimize the suffering of animals every necessary effort has been made. The experimental protocol of this study was approved by Kayseri Erciyes University Animal Experiments Local Ethics Committee (HADYEK). Mice weighted close to each other formed the experimental groups. According to the pentylenetetrazole (PTZ) incineration model, the animals were administered intraperitoneally (i.p.) PTZ chemical every 2 days. The level of epilepsy was recorded by observing the animals within thirty minutes after each injection.

Experimental groups

Control group (n=8): Rats were given 0.5 cc i.p. saline (SF) every 2 days for 1 month.

Untreated PTZ epilepsy group (n=8): PTZ is a chemical that causes severe seizures. Chronic sub-convulsive dosing of PTZ lowers the seizure threshold, inducing tonic-clonic seizures in animals. The PTZ-kindling model is a simple and widely applied method to investigate the pathophysiology of epilepsy with recurrent seizures (21,22). Recurrent PTZ-induced seizures may alter the balance of excitatory and suppressive neurotransmission and lead to seizure sensitivity. After the seizures, rats show hippocampal injury and reduced glucose metabolism like pilocarpine and kainic acid models (23). To induce chronic epilepsy in rats, PTZ-kindling protocol was applied as in our previous study. Briefly, PTZ (P6500, Sigma-Aldrich, St. Louis, MO, USA) dissolved in SF at a dose of 35 mg/kg was administered i.p. every 2 days for a month. Then, 50 mg/kg PTZ was injected to promote seizure susceptibility and mimic ictogenesis in animals (24).

TUDCA administered group (n=8): TUDCA, a hydrophilic bile acid, reduces apoptosis and exhibits suppressive effects on ER stress and oxidative stress. With these effects, the TUDCA compound shows neuronal protective activity in various neurodegenerative disorders like Alzheimer's disease and Amyotrophic Lateral Sclerosis (25,26). The TUDCA protocol was similarly performed at a dose that has been shown to be safe and effective from other studies (27,28). TUDCA (T0266 - Sigma-Aldrich,St. Louis, MO, USA) was dissolved in distilled water at a dose of 500 mg/kg and administered i.p. every two days for a month to rats.

PTZ epilepsy group (n=8) treated with TUDCA: To induce chronic epilepsy in rats, the epileptic agent 35 mg/kg PTZ was administered i.p. every two days for one month and a dose of 500 mg/kg TUDCA has been applied i.p. 30 minutes before PTZ administration. As part of this experimental model, PTZ chemical was injected i.p as 35 mg/kg. As a final dose, ictogenesis (clinical epileptic seizures) was imitated in animals with 50 mg/kg. All i.p. procedures were carried out at the same time of the day. After the experimental protocol was completed, ketamine

hydrochloride and 2% xylazine hydrochloride were administered to the rats to anesthetize, and then brain stem, heart and lung tissues were taken.

Histological procedure

Brain stem, lung and heart tissues taken from rats were fixed in 10% formaldehyde solution. Water recovery was applied by keeping the tissues in increasing alcohol series (70%, 80%, 90% and 100%) after washing in tap water (1 night). The tissues were kept in xylol and became transparent. These tissues were embedded in paraffin at the appropriate orientation. 5µm thick sections taken from tissue samples embedded in paraffin using a microtome were stained with Harris hematoxylin-eosin after that examined histomorphologically under a light microscope (Olympus BX53).

Immunohistochemical analysis

With Avidin-Biotin peroxidase method, anti-HIF1α (Santa Cruz Biotechnology, sc-13515), anti-TNF-α (Elabscience, E-AB-22159), anti-NF-κB p65 in the brain stem, heart and lung tissues of experimental groups (Bioassay Technology Laboratory, MT-BCA1291) and anti-Kir6.2 (Alomone labs, APC-020) proteins were detected immunoreactivity (29). In summary, after deparaffinization of 5µm thick sections, samples were heated in citrate buffer 2X4 times in a 300W microwave oven (pH: 6.0) to open epitopes. To inhibit endogenous peroxidase activity, preparations were then taken into a solution of 3% hydrogen peroxide in methanol. Ultra V block solution was applied to prevent non-specific staining. The sections were then incubated with primary antibodies at 4 0C overnight. Biotinylated secondary antibody, streptavidin-HRP, and DAB chromogens were applied, respectively after that sections were counterstained with Gill Hematoxylin. With a series of increasing alcohol and sealed with entellan, a sealant sections were dehydrated. Olympus BX53 light microscope is used to examine samples. TIFF images were imported into ImageJ software and the threshold function was applied to separate the signal from the background, and the average signal intensity was measured with the "measure" function to quantify the immunohistological staining for each protein (30-33). The mean intensity of the background was obtained by averaging the values of the negative control images treated with secondary antibody alone. The staining intensity level value gave the staining intensity level value divided by the average signal intensity above the background for a minimum of 10 images per mouse for a minimum of 3 mice per experimental group. (29)

Statistical analysis

Findings from immunohistochemical analyzes were evaluated with the Image-J Version 1.46 program. The data obtained from these results were determined with the Graphpad Prism 9.0 software package by applying one-way analysis of the variance test. The p value for statistical significance level was evaluated as < 0.05.

Results

TUDCA ameliorates the seizure-related histological injury observed in rats treated with chronic PTZ

Histological analysis of brain stem samples revealed that sections of PTZ group rats had enlarged blood vessels, bleeding areas, and apoptotic neurons compared to the TUDCA and control groups (Figure 1C). Brain stem sections from PTZ-TUDCA group rats, bleeding areas were not observed, but apoptotic neurons were found (Figure 1D).

The heart tissue sections taken from the control and TUDCA groups, especially the muscle cells in the myocardium layer, were arranged regularly and preserved their healthy histological content. However, we found that there were gaps/separations between muscle cells of PTZ group rats, and apoptotic cells with eosinophilic stained pycnotic nuclei were concentrated (Figure 1G). Examination of animals in the PTZ-TUDCA group revealed that the separation between muscle cells was significantly reduced and there were few apoptotic cells (Figure 1H).

Microscopic evaluation of lung tissue from control and TUDCA group animals showed the normal histology of bronchioles, alveolar and pulmonary arteries. In the lung sections of the PTZ group rats indicate that the integrity of the pulmonary arteries was impaired and there was an increase in inflammatory cell infiltration (Figure 1K). However, in the lung sections of the PTZ-TUDCA group, we observed that there was a normal histological structure of alveoli and pulmonary arteries similar to the control and TUDCA groups (Figure 1L). Examination of the brain stem, heart, and lung tissues indicates that TUDCA is effective in reorganizing and ameliorating PTZ-induced histopathological changes.

TUDCA reduces the expression of proteins associated with inflammation and hypoxia in the brainstem of epileptic rats

Immunohistochemical analysis of brain stem tissue; HIF1α, TNF-α, NF-kB p65 and Kir6.2 expressions were significantly increased in the PTZ and PTZ-TUDCA groups in comparison to the control group (Figure 2). Also, TUDCA administration markedly reversed the pathological effects of PTZ. TNF-α, NF-kB p65 and Kir6.2 levels were found to be significantly decreased 1.55 times, 1.56

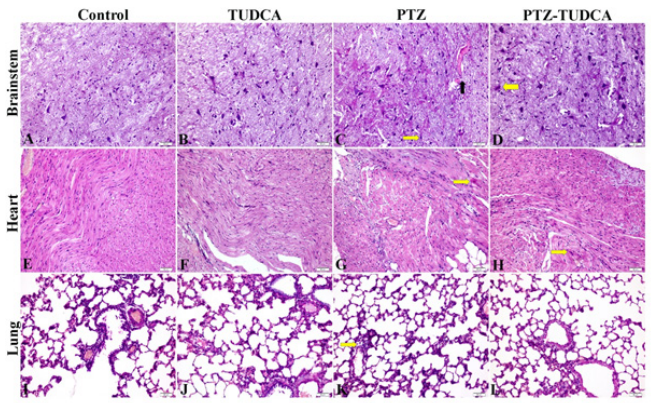

Figure 1. Hematoxylin-eosin staining images of brain stem, heart and lung tissues of rats. Tissues of control and TUDCA group animals show normal histological structure. Image of enlarged blood vessel and bleeding area (***black arrow***) in brain stem section of PTZ group rats. Apoptotic cells (***yellow arrow***) in brainstem and heart sections from PTZ and PTZ-TUDCA animals. The representative image of inflammatory cell infiltration (***yellow arrow***) in lung section of PTZ rat. Photomicrographs were taken at 20X magnification and a 50 µm scale bar.

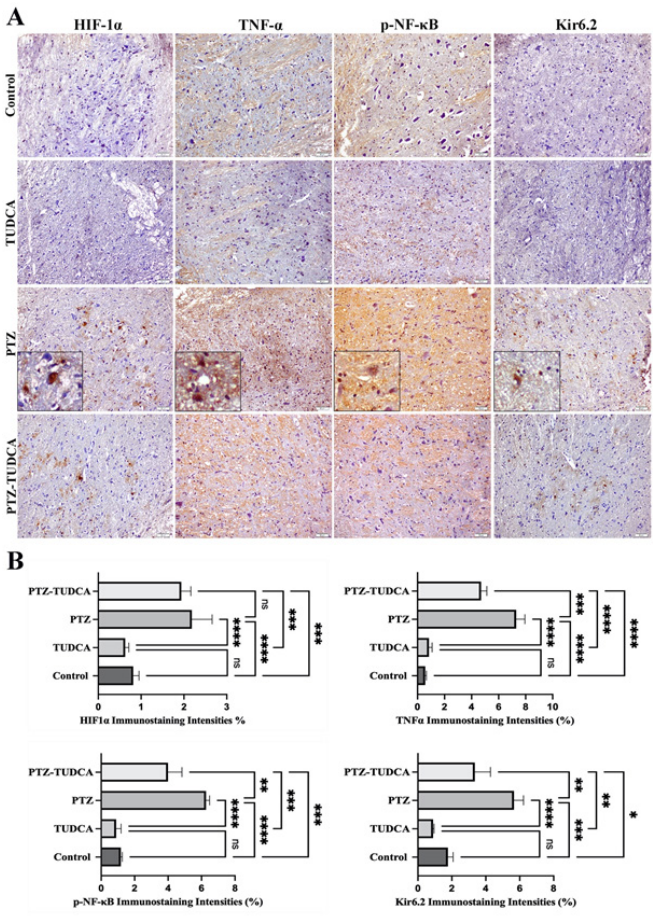

Figure 2. Immunostaining images of HIF1α, TNFα, NF-κB p65 and Kir6.2 proteins in brainstem tissues of rats. **A)** Localizations of the antibodies in the brain stem sections of the experimental groups. **B)** Histogram plots showing the immunoreactivity intensity of the antibodies analyzed. Data average is expressed as ± SEM. Tukey's multiple comparison test and one-way ANOVA analysis of variance were applied (*p<0.05, **p<0.01 ***p<0.001 and ****p<0.0001 indicates that there is a significant difference statistically and ns indicates that there is no statistically significant difference). Zoomed inserts were added, especially in PTZ groups where antibodies were more stained.

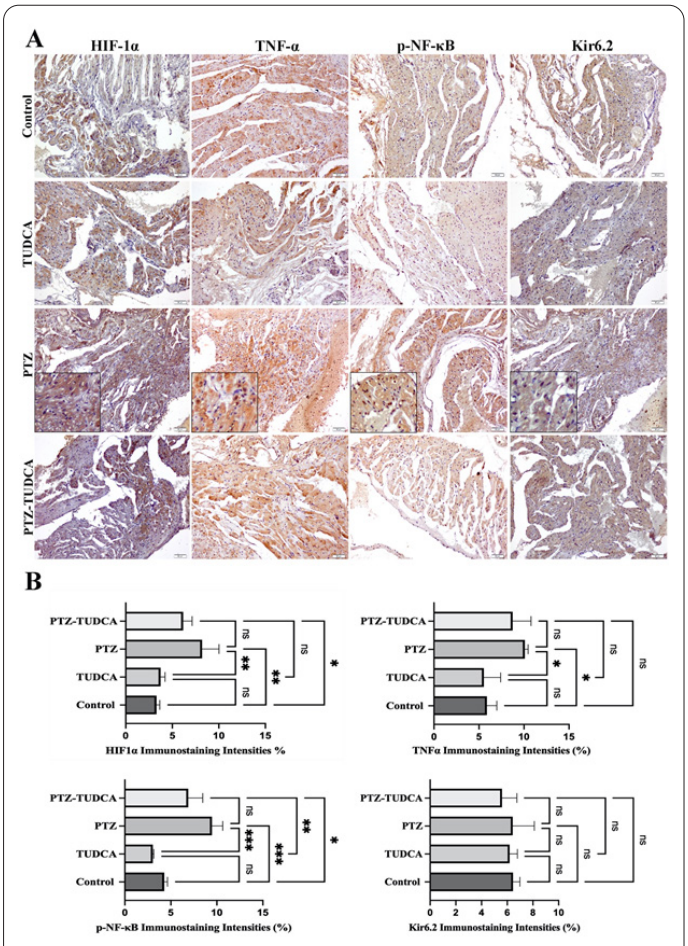

Figure 3. Immunostaining images of HIF1α, TNFα, NF-κB p65 and Kir6.2 proteins in atrial tissues of rats. **A)** Localizations of the antibodies in the atrium sections of the experimental groups. **B)** Histogram plots showing the immunoreactivity intensity of the antibodies analyzed. Data average is expressed as ± SD. Tukey's multiple comparison test and one-way ANOVA analysis of variance were applied (*p<0.05, **p<0.01 ***p<0.001 and ****p<0.0001 indicates that there is a significant difference statistically and ns indicates that there is a statistically significant difference and ns indicates that there is no statistically significant difference). Zoomed inserts were added, especially in PTZ groups where antibodies were more stained.

times and 1.68 times, respectively, in brainstem samples of PTZ-TUDCA group animals as compared with the PTZ group (Figure 2).

Administration of TUDCA reverses seizure-induced increase in immunoreactivity in ventricular sections of rats

HIF1α increased 2.48 times, TNF-α 1.71 times and NF-κB p65 2.21 times in atrium sections of epileptic rats from PTZ group but no significant change was found in Kir6.2 expressions compared with the control group. Also, no significant difference seen in immunoreactivity of the investigated proteins in the PTZ and PTZ-TUDCA groups (Figure 3).

In the ventricle sections of the PTZ group rats with seizures, the levels of examined proteins were increased compared to the control group. However, increasing protein expression were suppressed in the ventricles of TUDCA pretreated rats. Staining intensity of HIF1α (1.36 times), TNF-α (1.36 times), NF-kB p65 (1.71 times) and Kir6.2 (1.56 times) proteins in ventricular sections from PTZ-TUDCA group decreased compared to PTZ rats with seizures (Figure 4).

Inflammation and hypoxia-induced increased expression in lung tissues of epileptic rats are reduced by TUDCA treatment.

Immunohistochemical evaluation of lung sections of PTZ and PTZ-TUDCA group animals showed that HIF1α, TNF-α, NF-kB p65 and Kir6.2 protein levels were increased as compared with the control group. However, administration of TUDCA reduced the production of the proteins in the lung of chronic PTZ treated rats. These decreases were found to be 1.12 times for HIF1α, 1.55 times for TNF-α, 1.56 times for NF-κB p65, and 1.68 times for Kir6.2 (Figure 5). In addition, TUDCA administration did not affect the immunoreactivity of the proteins examined in brain stem, heart and lung samples under normal conditions.

Discussion

In our study, we showed that PTZ-induced seizures increase NF-κB p65, TNF-α, HIF1α and Kir6.2 channel immunoreactivity in relation to inflammation and hypoxia.

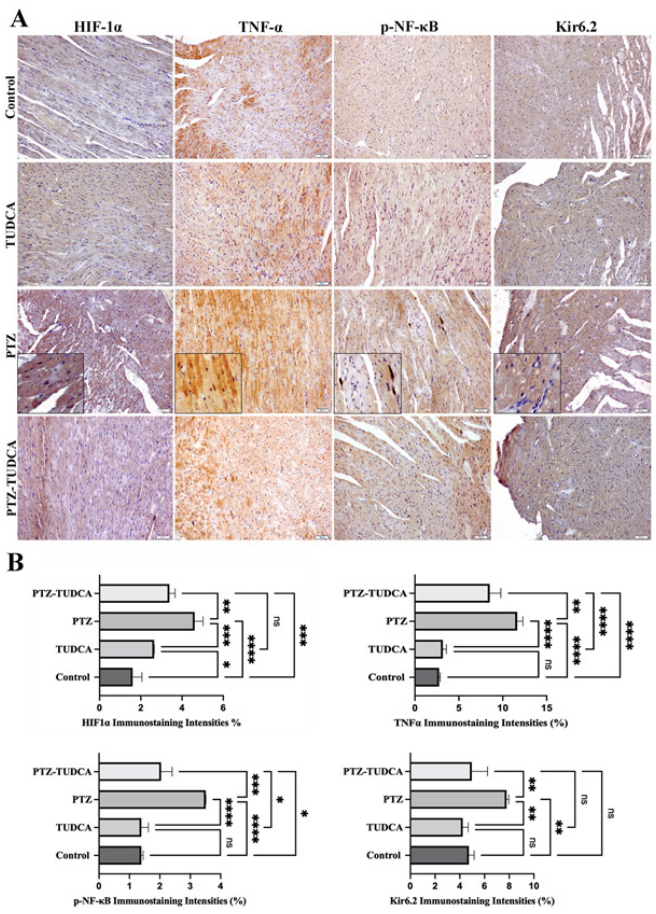

Figure 4. Immunostaining images of HIF1α, TNFα, NF-κB p65 and Kir6.2 proteins in ventricular tissues of rats. **A)** The localizations of the antibodies in the ventricular sections of the experimental groups. **B**) Histogram plots showing the immunoreactivity intensity of the antibodies analyzed. Data average is expressed as ± SD. Tukey's multiple comparison test and one-way ANOVA analysis of variance were applied (*p<0.05, **p<0.01 ***p<0.001 and ****p<0.0001 indicates that there is a significant difference statistically and ns indicates that there is a statistically significant difference and ns indicates that there is no statistically significant difference). Zoomed inserts were added, especially in PTZ groups where antibodies were more stained.

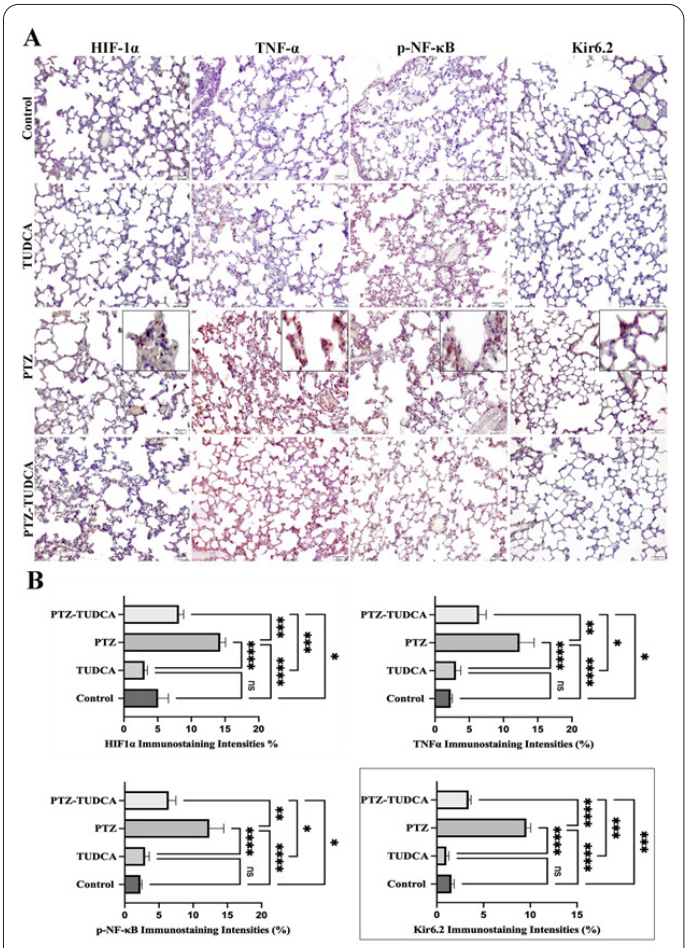

Figure 5. Immunostaining images of HIF1α, TNFα, NF-kB p65 and Kir6.2 proteins in lung tissues of rats. **A)** Localizations of the antibodies in the lung sections of the experimental groups. **B)** Histogram plots showing the immunoreactivity intensity of the antibodies analyzed. Data average is expressed as ± SD. Tukey's multiple comparison test and one-way ANOVA analysis of variance were applied (*p<0.05, **p<0.01 ***p<0.001 and ****p<0.0001 indicates that there is a significant difference statistically and ns indicates that there is no statistically significant difference). Zoomed inserts were added, especially in PTZ groups where antibodies were more stained.

Furthermore, we demonstrated that pre-treatment with TUDCA may play a protective role against heart and lung pathologies in rats with chronic seizures. In this context, our results indicate that ER stress caused by seizures may trigger respiratory and cardiac disorders by affecting inflammation and hypoxia-related pathways.

Analysis of brain tissues from the animal model of epilepsy revealed that production of the NF-κB p65 increased (34). In our study where autonomic network affected by seizures, we showed that NF-kB p65 expression was significantly increased in the brain stem, lung and heart tissues of epileptic rats compared to the control group. NF-κB p65 activation mediated death mechanisms, resulted in impaired left ventricular contraction and electrocardiographic abnormalities in the mouse model of arrhythmogenic cardiomyopathy and cell model formed from the ventricular heart muscle (35). Elevated NF-κB p65 expression increases apoptosis of cardiac muscle cells while decreases contraction (36). Left ventricular systolic/diastolic dysfunction has been observed in patients with generalized epilepsy and children with drug-resistant seizures (37,38). Accordingly, inflammation activated by the increase of NF-κB p65 transcription factor leading to cell death and structural damage may be one of the causal phenomena of seizure-related cardiac dysfunction. Suppression of inflammation in the brain stem was due to molecular inhibition of the NF-κB pathway in the experimental brain death model and reduce cardiovascular system deterioration (39). In this context, increased NF-κB p65 expression in the brainstem region may trigger the production of oxidative stress and inflammation that cause autonomic impairments.

In our study, we have noted a significant increase in TNF-α immunoreactivity in brain stem, heart (atria and ventricle) and lung tissues of PTZ-induced chronic epilepsy groups than control groups. These findings may indicate the importance of TNF-α in the mechanism of epilepsy. In the epilepsy kindling model created in the amygdala, rats treated with TNF-α showed prolonged epileptic discharge compared to the control group (40). This study may indicate that pro-inflammatory cytokines including TNF-α promote epileptiform activity. Inflammation in the hippocampus caused by TNF-α signaling triggers hyperexcitability and acute seizures in a mouse model of limbic epilepsy (41). Seizure injury could be mediated by TNF-

α-related inflammation. TNF-α levels in serum samples of pediatric epilepsy patients with acute seizure attacks are significantly increased as compared with control group (42). Accordingly, increased TNF-α ratio in tissues affected by seizures may trigger epilepsy-related autonomic disorders.

Data from literature reveal that HIF1α expression is increased in the brain tissue of epilepsy patients (43,44). Elevated HIF1α level may be a result of neuronal hypoxia because of increased oxygen consumption during epileptic seizures. In our study, we revealed that HIF1α production increased in autonomic system-related brain stem, lung and heart tissues as a result of PTZ-induced recurrent seizures compared to the control group. High HIF1α immunoreactivity in heart tissue is consistent with our studies evaluating cardiac HIF1α levels in pilocarpine or PTZ-induced seizures (45,46). In this context, HIF1α has a role in the pathogenesis of autonomic disorders accompanying seizures is suggested. The expression of pro-apoptotic ER stress marker and apoptotic molecules increased with the elevation of HIF1α expression in the lung and alveolar cells of rats exposed to hypoxia (47,48). Similarly, elevation of hypoxia-induced HIF1α has been associated with triggering of cardiomyocyte apoptosis in the mouse model of ischemia (49). Increased apoptosis and inflammation in the brain stem, heart and lung based on seizures may be the result of high HIF1α levels.

Kir6.2 channels have been reported to exert cytoprotective effects on neurons in hypoxia (50). We showed that PTZ-induced chronic epilepsy groups significantly increased Kir6.2 immunoreactivity in brainstem, heart (ventricle only), and lung tissues compared to control groups. Our data are consistent with our study showing increased Kir6.2 expression from medulla region of the brainstem in rats with chronic seizures (24). These findings may indicate the potential role of Kir6.2 in the mechanism of epileptic seizures leading to heart and lung disorders. In addition, Kir6 channels in the atrium and ventricles may differ in terms of structure. The pharmacological properties of the channels indicate a predominance of SUR1/Kir6.2 complexes in the atrium and SUR2A/Kir6.2 complexes in the ventricle (51). Electrophysiological recordings show that atrial and ventricular Kir6 channels exhibit different sensitivity to metabolic inhibition (52). In this context, SUR subtype complexing with Kir6.2 channels and varying sensitivity of atrial/ventricular Kir6 channels may explain the regional variation of the effect of seizures on Kir6.2 channel. Evaluation of the impact of epileptiform activity on SUR subunits and ion currents may contribute to the elucidation of possible mechanisms. However, 2-deoxy-D-glucose, a glycolytic suppressor, has been reported to exert anti-epileptic effects by upregulating the Kir6.2 channel in a pilocarpine-induced epilepsy model (53). Enhanced Kir6.2 immunoreactivity as a result of seizures may reflect the intrinsic defense mechanism of cells in autonomic structures to hypoxia. The adaptive respiratory responses induced in hypoxic conditions in mice in which the gene producing the Kir6.2 channel is deleted are less numerous and short-lived than in control mice (54). This situation may point to the importance of the Kir6.2 channel to retain healthy respiratory activity in seizure-induced hypoxia.

We obtained that TUDCA treatment decreased HIF1α expression in ventricular and lung tissues of rats with seizures. Our data may indicate that suppression of ER stress has a protective effect against autonomic disorders accompanying seizures. The absence of a substantial immunohistochemical change in the atria of PTZ-TUDCA group than control and PTZ groups revealed that the ventricles may be the center of the cardiac protective effect. However, in another study that supports our findings, increased susceptibility to PTZ-induced seizures in rats with repeated restraint stress decreases by TUDCA treatment (55). Similarly, in the pilocarpine-induced status epilepticus mouse model, TUDCA treatment was found to suppress inflammation-related molecular processes and ER stress (56). Genetic deletion of HIF1α in mice may lead to cardiac dysfunction by affecting the survival and function of sympathetic neurons (57). TUDCA do not significantly suppress the level of HIF1α in the brainstem. Therefore, HIF1α may be crucial for survival of neurons that have impact on cardiac and respiratory function. The expression of Kir6.2 is thought to be protective against seizures. Kir6.2 expressions was decreased in epileptic rats injected with TUDCA. This can be explained by the disappearance of hypoxic conditions leading to adaptive Kir6.2 increase as a result of suppression of ER stress. In addition, TUDCA treatment improved the features including inflammatory cell accumulation, formation of hemorrhagic areas, disruption of tissue integrity, and apoptosis. The suppression of pressure-induced cardiac apoptosis and fibrosis in mice with treated by TUDCA supports our histopathological findings (58). In neurological diseases including subarachnoid hemorrhage, traumatic brain injury and acute hemorrhagic stroke, TUDCA administration increases neuronal survival by suppressing apoptotic pathways (59-61) Interestingly, apoptotic neurons were seen in the brainstem of epileptic rats after TUDCA treatment. Similar study reported that HIF1α reduces neuronal apoptosis by suppressing caspase-3 activity in a rat model of cerebral ischemia (62,63). Accordingly, the presence of apoptotic cells in the brain stem of epileptic rats despite treatment may be related to the suppression of anti-apoptotic HIF1α by TUDCA treatment and the dual effect of HIF1α on neuronal survival. In summary, we demonstrated that TUDCA has immunohistochemical and histopathological protective effects against seizure-related cardiovascular and respiratory disorders. In epileptic rats, increased levels of NF-κB p65 and TNF-α may trigger elevation of cytokine levels and inflammatory cell accumulation that causes autonomic system related problems. Further studies may aim to examine the effects of TUDCA on HIF1α-related apoptotic downstream pathways at the molecular level.

Overall, natural products mediated health promoting effects and amelioration of disease pathologies has sparked interest in natural product research (64-66). Identification of most significant proteins and cellular pathways will be helpful for selection of most effective natural products for disease prevention.

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