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RESEARCH ARTICLE

Investigation of the Effects of Rutin on Sodium Valproate-Induced Lung Damage in Rats

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ABSTRACT

Sodium valproate (SVP) is a drug widely used in epilepsy, migraine, and bipolar disorders. In addition to its therapeutic properties, it has toxic effects on many organs in high doses and prolonged intake. Rutin flavonoid derivative is a natural antioxidant and has been successfully used in many toxications. In the present study, it was aimed to investigate the effects of rutin on SVP-induced lung injury. In the study, 35 Sprague Dawley rats were divided into five equal groups as control, routine, SVP, SVP+Rutin 50 and SVP+Rutin 100 and oral administration was continued for 14 days. At the end of the study, lung tissue was obtained and oxidative stress (MDA, GSH, SOD, CAT, GPx, Nrf-2, HO-1), endoplasmic reticulum stress (ATF-6, PERK), inflammation (NF-κB, TNF-α), apoptosis (Bax, Bcl-2, Caspase-3) and autophagy (Beclin-1) parameters were analyzed. The data obtained showed that SVP weakened the defense system by decreasing antioxidant enzyme activities and increased lipid peroxidation, inflammation, apoptosis, and autophagy, leading to increased cell damage. It was determined that SVP and rutin 50 and rutin 100 doses strengthened the antioxidant defense system, suppressed lipid peroxidation, endoplasmic reticulum stress, inflammation, apoptosis, and autophagy, and were effective in protecting the cell from damage. As a result, it was determined that rutin use was beneficial against SVP-induced lung injury.

Key Words: Lung damage, Rat, Rutin, Sodium valproate

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Ratlarda Sodyum Valproat Kaynaklı Akciğer Hasarı Üzerine Rutin'in Etkilerinin Araştırılması

ÖZ

Sodium valproat (SVP), başta epilepsi olmak üzere migren ve bipolar bozukluklarda yaygın kullanılan bir ilaçtır. Tedavi edici özelliği yanı sıra yüksek doz ve uzun süre alımlarda çoğu organda toksik etki göstermektedir. Rutin flavanoit türevi doğal bir antioksidandır ve birçok toksikasyonda başarı ile kullanılmıştır. Sunulan çalışmada SVP kaynaklı akciğer hasarı üzerine rutin' in etkilerinin araştırılması amaçlanmıştır. Çalışmada 35 Sprague Dawley rat kontrol, Rutin, SVP, SVP+Rutin 50 ve SVP+Rutin 100 olmak üzere beş eşit gruba ayrılarak 14 gün oral yolla uygulamalar devam etmiştir. Çalışma sonunda akciğer dokusu alınarak oksidatif stres (MDA, GSH, SOD, CAT, GPx, Nrf-2, HO-1), endoplazmik retikulum stresi (ATF-6, PERK), inflamasyon (NF-κB, TNF-α), apoptoz (Bax, Bcl-2, Caspase-3) ve otofaji (Beclin-1) parametreleri incelenmiştir. Elde edilen veriler SVP' nin antioksidan enzim aktivitelerini azaltarak savunma sistemini zayıflattığını, lipid peroksidasyonu, inflamasyonu, apoptozisi ve otofajiyi artırarak hücrede hasarın artmasına neden olduğu göstermiştir. SVP ile birlikte rutin 50 ve rutin 100 dozlarının antioksidan savunma sistemini güçlendirdiği, lipid peroksidasyonunu, endoplasmik retikulum stresini, inflamasyonu, apoptoz ve otofajiyi baskılayarak hücreyi hasardan korumada etkili olduğu tespit edildi. Sonuç olarak SVP kaynaklı akciğer hasarına karşı rutin kullanımının faydalı olduğu belirlendi.

Anahtar Kelimeler: Akciğer hasarı, Rat, Rutin, Sodyum valproat

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INTRODUCTION

Epilepsy is one of the most common neurological disorders. Intermittent, exaggerated, atypical episodes of cerebral activity lead to clinical features ranging from debilitating seizures, cerebral processing abnormalities, and mental health problems to the life-threatening clinical condition epilepticus syndrome (SE) (Al-Rafiah and Mehdar 2021). Sodium Valproate (SVP) is a histone deacetylase inhibitor that has been used for over 30 years to treat epilepsy, which affects 70 million people worldwide (Zhou et al. 2020; Korıglu et al. 2021). The mechanism of action of SVP is known to be inhibition of γ-aminobutyric acid (GABA) metabolism and interruption of GABA reuptake into nerve endings (Adewole et al. 2023). Therefore, SVP is used in the treatment of many psychiatric disorders such as migraine, bipolar disorder, and schizophrenia as well as epilepsy (Kandemir et al. 2022). Although SVP, a popular drug due to its therapeutic benefits and low cost, is well tolerated in the body, it has been reported to cause renal, hepatic, and pulmonary toxicity with cardiovascular, neurologic, and gastrointestinal side effects, and SVP use has been reported to cause widespread alveolar bleeding, especially in humans (Nanau and Neuman 2013; Öztay et al. 2020). Therefore, the reliability of SVP has been questioned by clinicians who initially supported it (Chaudhary et al. 2015). Although there are many reasons underlying the toxic effect of SVP, induction of oxidative stress and inflammation are among the most important ones. Therefore, research on natural active ingredients that regulate these mechanisms against SVP toxicity continues intensively (Akaras et al. 2023).

Foods contain certain compounds with effective antioxidant properties, such as flavonoids. These compounds are known as dietary antioxidants because of their beneficial properties. More than 4000 flavonoids, including rutin, have been evaluated as dietary antioxidants (Genç et al. 2019). Rutin is a flavonoid glycoside found predominantly in citrus fruits such as grapefruit, orange, lemon, spinach, onion, apple, buckwheat seeds, and tea. A potent scavenger of superoxide radicals, rutin's pharmacological properties include anti-inflammatory, antioxidant, antiallergic, and anticarcinogenic effects (Küçükler et al. 2021; Gür and Kandemir 2023).

The present study aimed to investigate the effects of rutin on SVP-induced lung injury with some biochemical parameters and examine the possible mechanisms of injury and the effect of rutin on these mechanisms.

MATERIALS and METHODS

Drug and Chemicals

SVP (Depakin, Sanofi, Turkey), Rutin Hydrate (≥ 94%, Sigma (R5143), USA), and other chemicals were of analytical purity and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Experimental Animals, Ethics Committee Aprproval and Experimental Design

In this study, 35 Sprague Dawley male rats from Ataturk University Animal Experimentation Center were used. The rats were kept in clean cages at a constant temperature of 24-25^{0}C and a 12-hour dark-light cycle. Water and feed were provided *ad-libitum*. The ethics committee approval of the study was obtained from Ataturk University Animal Experiments Local Ethics Committee with the meeting number 2023/14 and decision number 212 on 25.12.2023.

Five different groups of seven rats each were formed in the experiment. Rutin and SVP doses were determined with reference to previous studies (Kandemir et al. 2022; Akaras et al., 2023).

Group 1 (Control): Rats were given oral saline every day for 14 days.

Group 2 (Rutin): Rats received 100 mg/kg dose of rutin orally for 14 days.

Group 3 (SVP): Rats received SVP at a dose of 500 mg/kg orally for 14 days.

Group 4 (SVP+Rutin 50 mg/kg): Rats were orally administered SVP at a dose of 500 mg/kg for 14 days and 50 mg/kg of rutin was orally administered 30 minutes later.

Group 5 (SVP+Rutin 100 mg/kg): Rats were given SVP at a dose of 500 mg/kg orally for 14 days and 30 minutes later, 100 mg/kg of rutin was given orally.

24 hours after the last rutin treatment, rats were decapitated under mild sevoflurane anesthesia, lung tissues were removed and stored at -80 °C until biochemical analysis.

Analyzes of Oxidative Stress Parameters

Lung tissue was ground with liquid nitrogen (Tissue Lyser II, Qiagen) and homogenized in 1.15% potassium chloride buffer at a ratio of 1:10 (weight/volume). A portion of the homogenate was centrifuged at 10,000 rpm for 20 minutes at 4°C and the supernatant obtained was used to measure glutathione peroxidase (GPx) activity and glutathione (GSH) level. The remaining homogenate was centrifuged at 3500 rpm for 15 minutes and the supernatants were used for catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) analysis. GPx activity was measured according to Matkovics (1988), CAT activity according to Aebi (1984), SOD activity according to Sun et al. (1988), MDA level according to Placer et al. (1966) and GSH level according to Sedlak and Lindsay (1968). Total protein in the homogenate was determined according to the method of Lowry et al. (1951).

RT-PCR analysis

To analyze the relative mRNA transcript levels of the genes whose primer sequences are given in Table 1 in lung tissues, total RNA was first isolated from the tissues with QIAzol Lysis Reagent (79306; Qiagen). Then, cDNAs were synthesized from total RNAs with iScript cDNA Synthesis Kit (Bio-Rad). In the last step, cDNAs were reacted with primers of the relevant genes and iTaq Universal SYBR Green Supermix (BIORAD) in Rotor-Gene Q (Qiagen). After the reaction was completed, genes were normalized to β-actin using the 2-deltadeltaCT method (Livak and Schmittgen 2001).

Table 1: Primer sequences

Gene	Sequences (5'-3')	Length (bp)	Accession No
Nrf2	F: TTTGTAGATGACCATGAGTCGC	161	NM_031789.2
	R: TCCTGCCAAACTTGCTCCAT		
HO-1	F: ATGTCCCAGGATTTGTCCGA	144	NM_012580.2
	R: ATGGTACAAGGAGGCCATCA		
ATF-6	F: TCAACTCAGCACGTTCCTGA	130	NM_001107196.1
	R: GACCAGTGACAGGCTTCTCT		
PERK	F: GATGCCGAGAATCATGGGAA	198	NM_031599.2
	R: AGATTCGAGAAGGGACTCCA		
NF-κB	F: AGTCCCGCCCCTTCTAAAAC	106	NM_001276711.1
	R: CAATGGCCTCTGTGTAGCCC		
TNF-α	F: CTCGAGTGACAAGCCCGTAG	139	NM_012675.3
	R: ATCTGCTGGTACCACCAGTT		
Caspase-3	F: ACTGGAATGTCAGCTCGCAA	270	NM_012922.2
	R: GCAGTAGTCGCCTCTGAAGA		
Bcl-2	F: GACTTTGCAGAGATGTCCAG	214	NM_016993.2
	R: TCAGGTACTCAGTCATCCAC		
Bax	F: TTTCATCCAGGATCGAGCAG	154	NM_017059.2
	R: AATCATCCTCTGCAGCTCCA		
Beclin-1	F: TCTCGTCAAGGCGTCACTTC	198	NM_053739.2
	R: CCATTCTTTAGGCCCCGACG		
β-Actin	F: CAGCCTTCCTTCTTGGGTATG	360	NM_031144.3
	R: AGCTCAGTAACAGTCCGCCT		

One-way analysis of variance (ANOVA) and Tukey post hoc test (version 20.0; SPSS, Chicago, IL) were used to determine the differences between the groups and their significance levels. $p<0.05$ was considered a significant difference. All values were expressed as mean±standard derivation of the mean (SD).

RESULTS

Oxidative Stress

When lung tissue MDA levels were examined (Figure 1A), it was found that there was no difference between the control group and the rutin group ($p>0.05$), SVP administration increased the MDA level approximately 2-fold compared to the control and rutin groups ($p<0.001$), rutin 50 dose administered with SVP was not effective in reducing the MDA level, while rutin 100 dose successfully reduced the MDA level ($p<0.05$). In addition, GSH level (Figure 1B) and SOD (Figure 1C), CAT (Figure 1D) and GPx (Figure 1E) activities did not differ between the control and rutin groups ($p>0.05$), SVP administration caused a significant decrease in these antioxidant enzymes ($p<0.001$), and doses of rutin 50 and rutin 100 administered together with SVP caused a gradual and dose-dependent increase in antioxidant activities ($p<0.05$) and protected the lung tissue from oxidative damage.

When Nuclear released factor-2 (Nrf-2, Figure 2A) and Heme oxygenase-1 (HO-1, Figure 2B) mRNA expression levels, which strengthen the defense system by increasing the expression of antioxidant enzymes, were examined, it was determined that there was no difference between the control and rutin groups ($p>0.05$), SVP administration caused a decrease in Nrf-2 and HO-1 mRNA expression levels ($p<0.001$), while rutin 50 and 100 doses gradually increased these levels ($p<0.005$).

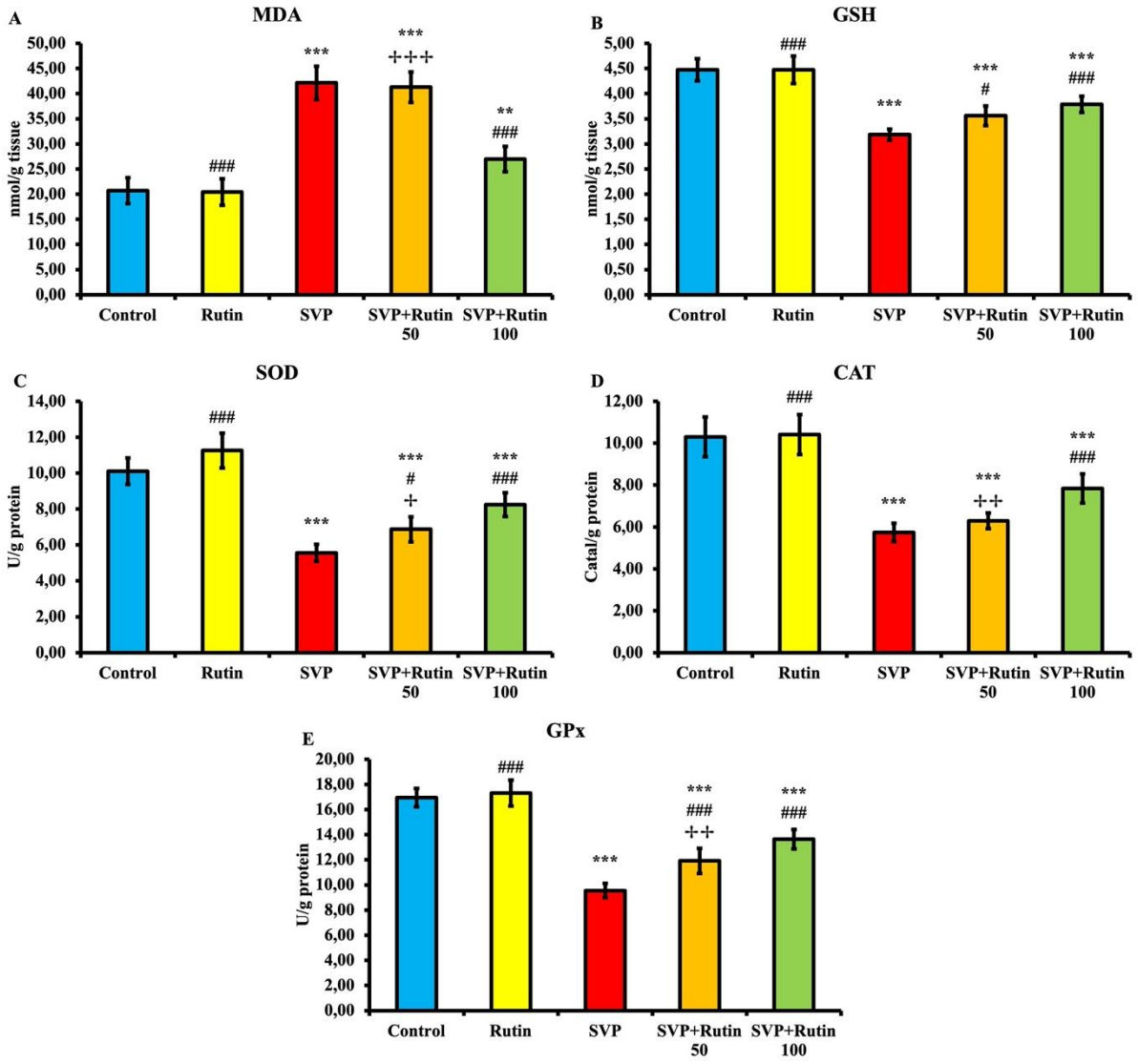

Figure 1: Lung tissue MDA (A) and GSH (B) levels and SOD (C), CAT (D) and GPx (E) activities after SVP and Rutin applications to rats. Statistical significance; Control and others: * p<0.05, ** p<0.01, *** p<0.001, SVP and orhers: # P < 0.05, ## p<0.01, ### p<0.001, SVP + Rutin 50 ve SVP + Rutin 100: ✢ P < 0.05, ✢✢ p<0.01, ✢✢✢ p<0.001.

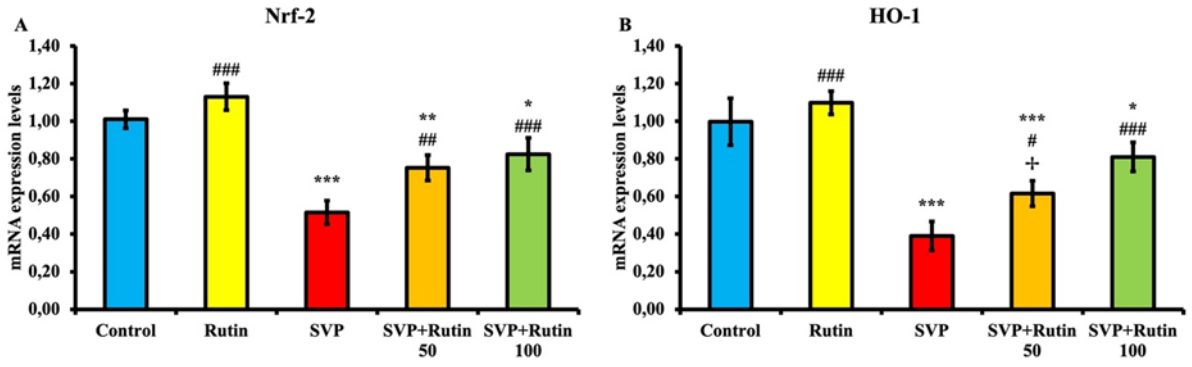

Figure 2: Lung tissue Nrf-2 (A) and HO-1 (B) mRNA expression levels after SVP and Rutin applications to rats. Statistical significance; Control and others: * p<0.05, ** p<0.01, *** p<0.001, SVP and others: # P < 0.05, ## p<0.01, ### p<0.001, SVP + Rutin 50 ve SVP + Rutin 100: ✢ P < 0.05, ✢✢ p<0.01, ✢✢✢ p<0.001.

Endoplasmic Reticulum Stress

When the activities of Transcription activating factor 6 (ATF-6, Figure 3A) and Protein kinase-like endoplasmic reticulum kinase mRNA expression levels (PERK, Figure 3B), which are among the endoplasmic reticulum stress parameters, were examined, it was found that there was no difference between the control and rutin groups in terms of ATF-6 and PERK mRNA expression levels ($p<0.05$), SVP treatment caused an increase in both parameters compared to control and rutin groups ($p<0.001$), rutin 50 and rutin 100 doses were effective in reducing endoplasmic reticulum stress by affecting ATF-6 and PERK mRNA expression levels ($p<0.05$).

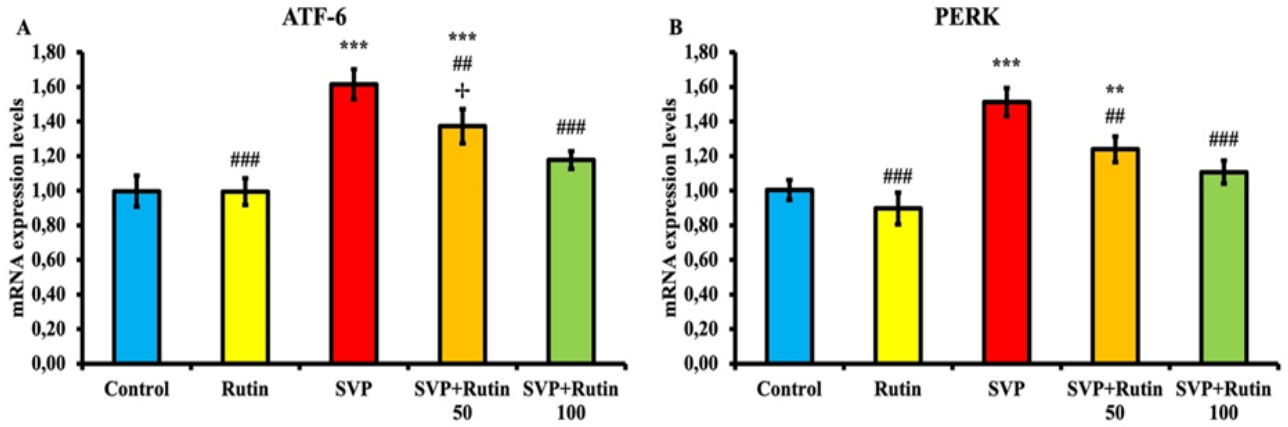

Figure 3: Lung tissue ATF-6 (A) and PERK (B) mRNA expression levels after SVP and Rutin applications to rats. Statistical significance; Control and others: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, SVP and others: # $P < 0.05$, ## $p<0.01$, ### $p<0.001$, SVP + Rutin 50 ve SVP + Rutin 100: + $P < 0.05$, ++ $p<0.01$, +++ $p<0.001$.

Inflammation

When Nuclear factor kappa B (NF-κB, Figure 4A) and Tumor necrosis factor alpha (TNF-α, Figure 4B) mRNA expression levels, which are among the most important markers of inflammation, were examined, SVP administration significantly increased NF-κB and TNF-α mRNA expression levels compared to control and rutin groups ($p<0.001$), while rutin 50 and rutin 100 doses given together with SVP suppressed inflammation by gradually and dose-dependently decreasing NF-κB and TNF-α levels ($p<0.05$).

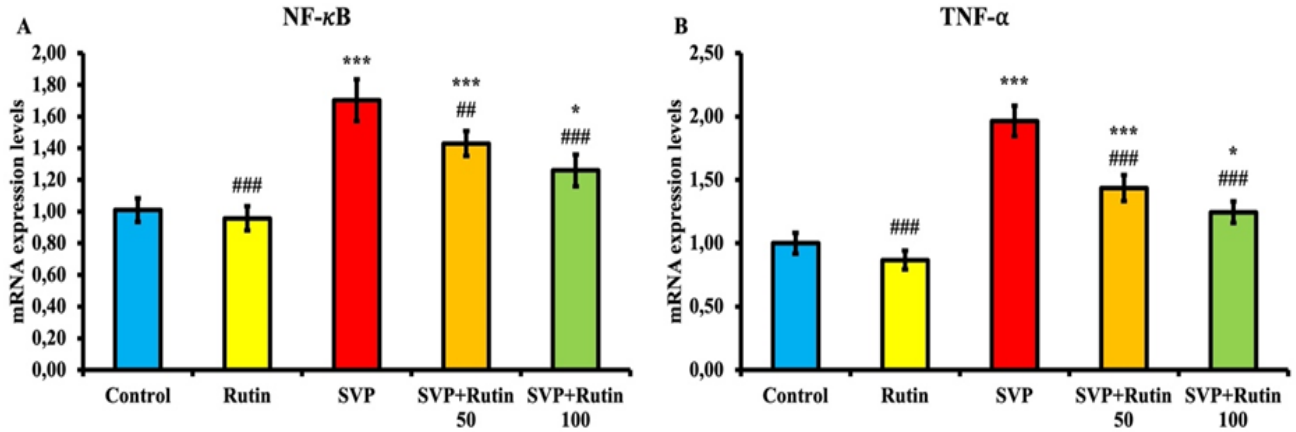

Figure 4: Lung tissue NF-κB (A) ve TNF-α (B) mRNA expression levels after SVP and Rutin applications to rats. Statistical significance; Control and others: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, SVP and others: # $P < 0.05$, ## $p<0.01$, ### $p<0.001$, SVP + Rutin 50 ve SVP + Rutin 100: + $P < 0.05$, ++ $p<0.01$, +++ $p<0.001$.

Apoptosis

The levels of Bcl-2-related x protein (Bax, Figure 5A), B-cell lymphoma gene-2 (Bcl-2, Figure 5B), and Cysteine aspartate-specific protease-3 mRNA expression levels (Caspase-3, Figure 5A), which are important markers of apoptosis, were analyzed. It was determined that proapoptotic marker Bax and apoptotic Caspase-3 mRNA expression levels were significantly increased with SVP treatment compared to control and rutin groups ($p<0.001$), while antiapoptotic marker Bcl-2 level was decreased with SVP treatment ($p<0.001$) and apoptosis was accelerated in the cells. Rutin 50 and 100 doses administered together with SVP were found to be successful ($p<0.05$) in suppressing apoptosis by decreasing Bax and caspase-3 and increasing Bcl-2 mRNA expression levels.

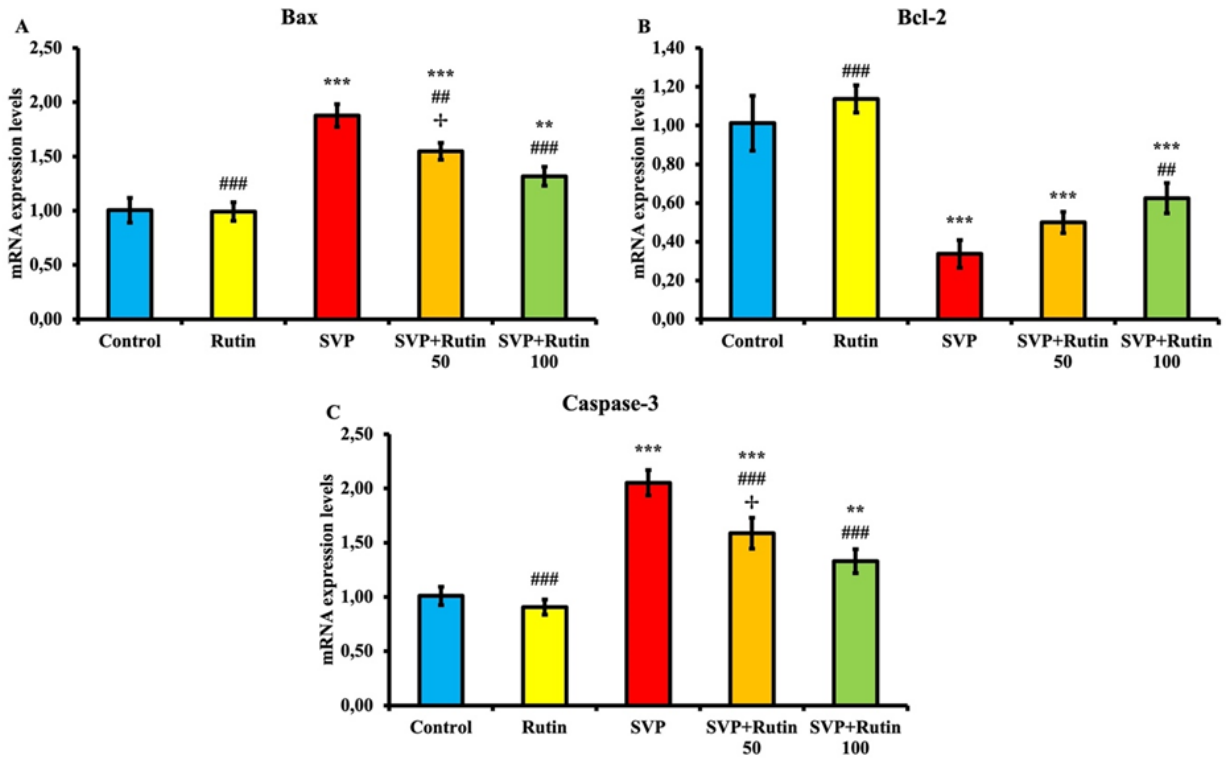

Figure 5: Lung tissue Bax (A), Bcl-2 (B) and Caspase-3 (C) mRNA expression levels after SVP and Rutin applications to rats. Statistical significance; Control and others * p<0.05, ** p<0.01, *** p<0.001, SVP and others: # P < 0.05, ## p<0.01, ### p<0.001, SVP + Rutin 50 ve SVP + Rutin 100: ✢ P < 0.05, ✢✢ p<0.01, ✢✢✢ p<0.001.

Autophagy

When Beclin-1 level, the most significant autophagy marker, was examined (Figure 6), it was found that there was no difference between the control and rutin groups (p>0.05), SVP administration caused an increase in lung tissue Beclin-1 mRNA expression levels (p<0.001), and both rutin 50 and 100 doses administered together with SVP were effective in suppressing autophagy by decreasing Beclin-1 mRNA expression levels (p<0.05).

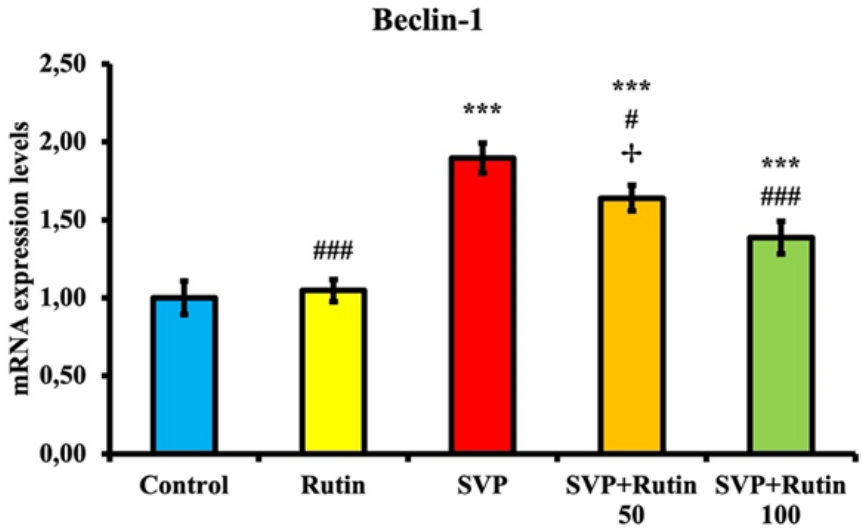

Figure 6: Lung tissue Beclin-1 mRNA expression levels after SVP and Rutin applications to rats. Statistical significance; Control and others:: * p<0.05, ** p<0.01, *** p<0.001, SVP and others: # P < 0.05, ## p<0.01, ### p<0.001, SVP + Rutin 50 ve SVP + Rutin 100: ✢ P < 0.05, ✢✢ p<0.01, ✢✢✢ p<0.001.

DISCUSSION

SVP is a well-known anti-epileptic drug and is also used to control convulsions, bipolar disorders and migraines (Gheena et al. 2022). In addition to its therapeutic properties, it has been reported to cause various organ damages including lung toxicity in long-term and high dose intake (Öztay et al.2020). Therefore, in the present study, the effects of rutin on sodium valproate-induced lung injury were investigated.

One of the main mechanisms of SVP toxicity is oxidative stress (Akarsu et al. 2023). Increased reaktive oxygene species (ROS) results in oxidative stress, which has a direct effect on acute and chronic lung injury. Increased ROS levels can directly cause tissue damage and promote inflammatory responses through the regulation of various pro-inflammatory mediators in the lungs (Akcılar et al. 2015a). It has been reported that there is excessive intracellular stress due to the process of peroxidation of membrane lipids and accumulation of free radicals, decreased enzymatic and non-enzymatic antioxidants, and increased MDA levels, and this is associated with metabolites derived from SVP. It was also reported that the decrease in intracellular antioxidants may be due to the overuse of antioxidants to neutralize the metabolites of SVP (Gheena et al. 2022). The organism has developed various ways to protect itself from the negative effects of oxidative attacks (Keleş et al. 2014; Ekinci-Akdemir et al. 2019). These mechanisms include various antioxidant enzymes such as SOD, CAT, GPx and non-enzymatic antioxidants such as GSH (Aydın et al. 2009; Kandemir et al. 2018; Kuzu et al. 2021; Kandemir et al. 2021). In the present study, it was determined that MDA levels in lung tissue of SVP-treated rats increased, SOD, CAT, and GPx activities and GSH levels decreased and oxidative stress developed in the cell. In addition, it was determined that rutin 50 and 100 doses administered with SVP decreased MDA levels and increased SOD, CAT, and GPx activities and GSH levels, strengthening the antioxidant system and protecting the cell from damage caused by oxidative stress. In studies conducted on the subject and targeting mechanisms in different organs, it was determined that SVP disrupted the cell membrane structure by increasing MDA levels and caused a decrease in antioxidant levels, and it was reported that rutin application was effective in reducing oxidative stress (Akaras et al. 2023a; Akarsu et al. 2023a, Kandemir et al. 2020).

The Nrf-2-antioxidant response element (ARE) system is stimulated by oxidative stress and plays an important role in tissue regeneration by regulating the expression of antioxidant and anti-inflammatory proteins in living organisms (Kocak et al. 2016). One of the target genes of Nrf-2 is HO-1. HO-1 oxidatively cleaves heme into biliverdin and carbon monoxide, thus eliminating the pro-oxidant effects of heme (Semiş et al. 2022). Activation of the Nrf-2 signaling pathway can effectively prevent oxidative stress-induced damage and contribute to tissue healing (Şimşek et al. 2023a; Tuncer et al. 2023a). In the present study, it was determined that SVP application caused a decrease in Nrf-2 and HO-1 mRNA experssion levels similar to antioxidant enzyme activities, and rutin application stimulated the Nrf-2 signaling pathway increased HO-1 mRNA experssion levels and decreased oxidative stress-induced damage. Nrf-2 and HO-1 have been examined as target parameters in many studies, it was reported that they were suppressed by different chemical agents, antioxidant enzyme activities decreased and the cell entered into oxidative stress effect, while flavonoids were found to increase antioxidant enzyme activities by stimulating Nrf-2 and HO-1 gene expression (Şimşek et al. 2023b; Gür et al. 2023; Çomaklı et al. 2023; Kankılıç et al. 2024a).

One of the mechanisms triggered by oxidative stress is endoplasmic reticulum (ER) stress (Semiş et al. 2021). The endoplasmic reticulum is an organelle involved in protein synthesis, folding and maturation, post-translational mechanisms, and calcium homeostasis (Ileriturk et al. 2023). Physiological and environmental factors contribute to the accumulation of unfolded and misfolded proteins in the ER lumen, as well as Ca imbalance. As a result, cells develop an unfolded protein response (UPR). As the stress persists, the UPR continues to elongate, ER stress develops and the cell undergoes apoptosis (Akaras et al. 2023b). It was reported by Chen et al. (2000) that SVP triggers ER stress by increasing oxidative stress. In the present study, SVP administration was found to increase ATF-6 and PERK mRNA experssion levels in lung tissue and thus ER stress. Rutin administration together with SVP was found to be effective in decreasing ATF-6 and PERK mRNA experssion levels and suppressing ER stress. It has been reported in different studies that rutin is effective in reducing ER stress and this effect is primarily achieved by reducing oxidative stress (Kandemir et al. 2022; Gür and Kandemir 2023).

Oxidative stress is also a factor in inflammatory damage (Şimşek et al. 2023c; Aksu et al. 2018). It is known that inflammation, another mechanism in the progression of lung injury, plays an important role and oxidative stress is one of the triggers of the inflammatory process in lung tissue. Inflammatory cytokines and chemokines are released by lung cells in association with oxidative stress. NF-κB is one of the main regulatory transcription factors regulating inflammatory responses and regulates proinflammatory cytokines such as TNF-α (Yeşildağ et al. 2022). TNF-α initiates and regulates the cytokine cascade in the cellular immune response process (Akarsu et al. 2023b). Different studies have shown that SVP administration accelerates inflammation by increasing NF-κB and TNF-α levels (Abu-Risha et al. 2024; Akaras et al. 2023a, Akarsu et al. 2023a). In the

present study, it was determined that SVP administration increased lung tissue NF-ϰB and TNF-α levels, and both doses of rutin administered together with SVP were effective and suppressed inflammation. It has also been demonstrated in different studies that rutin suppresses inflammation by decreasing NF-ϰB and TNF-α levels and shows anti-inflammatory properties (Küçükler et al. 2021; Tuncer et al. 2023b).

ROS are largely produced in mitochondria and have a significant impact on the development of apoptosis (Şimşek and Akaras, 2023). Apoptosis is a programmed cell death process that plays a role in the elimination of damaged cells under normal conditions (Akcılar et al. 2015b). In healthy tissues, apoptosis plays an important role in removing damaged or dangerous cells from the body (Kankılıç et al. 2024b). However, failure to control apoptosis causes damage in various tissues. Bcl-2 and caspase family proteins have an important role in the regulation of apoptosis. It is well known that oxidative stress mediates apoptosis by interacting with Bcl-2 and caspase family proteins (Yıldız et al. 2022). Caspases are inactive in cells, and when a caspase is activated, it initiates a cascade to activate other pro-caspases (Şimşek at al. 2016). The Bcl-2 family consists of pro-apoptotic and anti-apoptotic proteins that determine cell survival decisions. Bax in this family triggers apoptosis, while Bcl-2 is responsible for inhibiting apoptosis. The balance between these proteins determines the fate of cells in the apoptotic pathway. When an increase in the Bax/Bcl-2 ratio occurs, caspase 9 is activated. Activated Caspase-9 increases caspase 3 expression and apoptosis is triggered (Tabeshpour et al. 2020; Ekinci-Akdemir et al. 2022). It has been shown in many studies that SVP accelerates apoptosis by increasing Bax and caspase-3 levels and decreasing Bcl-2 levels in different organs, while rutin administration is effective in reducing apoptosis, especially decreasing the Bax/Bcl-2 ratio (Akaras et al. 2023a; Akarsu et al. 2023; Kandemir et al. 2022). In the present study, it was determined that Bax and Caspase-3 mRNA expression levels increased, Bcl-2 levels decreased and apoptosis accelerated in the SVP-treated group. Rutin administration together with AVP was found to be effective in reducing apoptosis at both doses.

Autophagy is a highly conserved physiological process that involves the recycling of proteins and molecules in the cytoplasm within autophagolysosomes (Kanlılıç et al. 2024c). When autophagy mediated by oxidative stress occurs at high levels, it triggers cell death and causes loss of function in tissues (Gür et al. 2022). One of the important markers in autophagy is Beclin-1 (Kanlılıç et al. 2024c). Beclin-1 is an essential protein involved in many important biological processes such as immunity, development, and tumor suppression (Tuncer et al. 2023c). In the present study, it was determined that there was a significant increase in Beclin-1 mRNA expression levels in the lung tissue of rats administered SVP and SVP-triggered autophagy. It was determined that rutin given together with SVP was effective at both doses and suppressed autophagy by decreasing the Beclin-1 mRNA expression levels. The anti-autophagic properties of rutin have been demonstrated in studies conducted in different tissues (Akaras et al. 2023a; Akarsu et al. 2023a).

CONCLUSION

When the data obtained in the study were evaluated, it was determined that SVP administration to rats caused lung tissue damage by increasing oxidative stress, ER stress, inflammation, apoptosis and autophagy, Rutin administration reduced this damage by showing antioxidant, anti-inflammatory, anti-apoptotic, and anti-autophagic effects, and as a result, the use of rutin in SVP-induced lung damage was beneficial.

Conflict of interest: The authors have no conflicts of interest to report.

Author' s Contributions: ÖK and CG contributed to the experimental design, biochemical analysis. ÖK drafted and wrote the manuscript. ÖK and CG reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Ataturk University Reserch Animals Application Center. This research was approved by The Ethics Committee of the Ataturk University (meeting number 2023/14, dated 25.12.2023 and decision number 212)

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