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Pulmonary toxicity associated with high-dose favipiravir and treatment options: biochemical and histopathological evaluation.

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Keywords: Favipiravir; lacidipine; thiamine pyrophosphate; adenosine triphosphate; oxidative stress.

Abstract. Favipiravir is a broad-spectrum antiviral drug that is a viral RNA-dependent RNA polymerase inhibitor. Favipiravir is used in high doses to treat COVID-19 but has a side effect on humans at high doses. The side effects of favipiravir have been associated with oxidative stress in the literature. In this trial, we investigated the biochemical and histopathological effects of lacidipine, thiamine pyrophosphate (TTP), and adenosine triphosphate (ATP), drugs with antioxidant properties, on the lung toxicity caused by high-dose favipiravir in rats. The rats were classified into five groups: healthy (HG), favipiravir alone (Fav), lacidipine+favipiravir (LFav), TPP+favipiravir (TFav), and ATP+favipiravir (AFav). Favipiravir (800 mg/kg) was administered twice daily for seven days. Lacidipine (4 mg/kg), TPP (20 mg/kg), and ATP (25 mg/kg) were administered once daily for seven days. Oxidant (malondialdehyde), non-enzymatic (total glutathione), and enzymatic (superoxide dismutase and catalase) antioxidant levels were measured in the excised lung tissues. Furthermore, the tissues were histopathologically examined. The systemic administration of high doses of favipiravir increased oxidant levels and decreased antioxidant levels in the lung tissue of rats.

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In parallel, the histopathological examination of the lung tissue revealed the presence of severe mononuclear cell infiltrations in interstitial areas and pronounced lymphoid hyperplasia. Lacidipine exhibited superior efficacy in mitigating oxidative stress and preventing the decline of antioxidants induced by favipiravir compared with TPP and ATP. Histopathologically, the lacidipine administration significantly reduced lung oxidative damage. TTP moderately reduced severe favipiravir-associated lung injury. However, ATP was ineffective against favipiravir-associated lung injury. Lacidipine offers more therapeutic benefits than TPP in treating oxidative lung injury caused by high doses of favipiravir.

Toxicidad pulmonar asociada a altas dosis de favipiravir y opciones de tratamiento: Evaluación bioquímica e histopatológica.

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Palabras clave: Favipiravir; lacidipino; pirofosfato de tiamina; trifosfato de adenosina; estrés oxidativo.

Resumen. El Favipiravir es un fármaco antiviral de amplio espectro que es un inhibidor de la ARN polimerasa viral dependiente de ARN. El Favipiravir se usa en dosis altas para tratar el COVID-19, pero tiene efectos secundarios en humanos a estas dosis. Los efectos secundarios del favipiravir se han asociado con el estrés oxidativo en la literatura. En este trabajo experimental, investigamos los efectos bioquímicos e histopatológicos de lacidipina, pirofosfato de tiamina (TTP) y trifosfato de adenosina (ATP), fármacos con propiedades antioxidantes, sobre la toxicidad pulmonar causada por altas dosis de favipiravir en ratas. Las ratas se clasificaron en cinco grupos: sanas (HG), favipiravir solo (Fav), lacidipina+favipiravir (LFav), TPP+favipiravir (TFav) y ATP+favipiravir (AFav). Se administró favipiravir (800 mg/kg) dos veces al día durante siete días. Se administraron lacidipina (4 mg/kg), TPP (20 mg/kg) y ATP (25 mg/kg) una vez al día durante siete días. Se midieron los niveles de antioxidantes oxidantes (malondialdehído), no enzimáticos (glutatión total) y enzimáticos (superóxido dismutasa y catalasa) en los tejidos pulmonares disecados. Además, los tejidos fueron examinados histopatológicamente. La administración sistémica de altas dosis de favipiravir aumentó los niveles de oxidantes y disminuyó los niveles de antioxidantes en el tejido pulmonar de ratas. Paralelamente, el examen histopatológico del tejido pulmonar reveló la presencia de graves infiltraciones de células mononucleares en las zonas intersticiales y una pronunciada hiperplasia linfoide. Lacidipina mostró una eficacia superior para mitigar el estrés oxidativo y prevenir la disminución de antioxidantes inducida por favipiravir en comparación con TPP y ATP. Histopatológicamente, la administración de lacidipina redujo significativamente el daño oxidativo pulmonar. La TTP redujo moderadamente la lesión pulmonar grave asociada al favipiravir. Sin embargo, el ATP fue ineficaz contra la lesión pulmonar asociada al favipiravir. Lacidipina ofrece más beneficios terapéuticos que el TPP en el tratamiento de la lesión pulmonar oxidativa causada por altas dosis de favipiravir.

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INTRODUCTION

Favipiravir is a nucleoside-derived prodrug with a wide range of antiviral activity. It acts by inhibiting the viral RNA-dependent RNA polymerase (RdRp) [1] and has been used to treat viral infections, such as Ebola and severe acute respiratory syndrome (SARS-CoV-2) [2]. Furthermore, favipiravir is approved to be used as an antiviral medication in Japan to treat influenza virus infections [3]. The effectiveness of favipiravir against influenza has been verified via cell cultures, animal studies, and clinical trials [4]. Additionally, favipiravir has been undergoing clinical trials as an investigational drug owing to its potential application in treating the novel coronavirus disease 2019 (COVID-19) [5]. The results of preclinical and clinical investigations indicate that favipiravir shows promise as a potential treatment option for severe infections caused by human rhinovirus, respiratory syncytial virus, metapneumovirus, parainfluenza viruses, and hantavirus pulmonary syndrome [4]. In addition, favipiravir has been reported to be a promising and effective antiviral medication for treating patients with COVID-19 [3]. For COVID-19 treatment, a loading dose of 2400–3000 mg (given in two doses) every 12 h followed by a maintenance dose of 1200–1800 mg every 12 h was recommended [6,7]. Numerous studies have evaluated favipiravir's effectiveness and potential side effects in treating patients with COVID-19 [8]. High doses of favipiravir have been associated with severe side effects in humans [9]. Additionally, signs of toxicity have been observed in animals administered with high doses of favipiravir [10]. Although the lethal dose of favipiravir in animals is >2000 mg/kg, it is administered to patients at higher doses, such as 6000 mg/day on the first day and 2400 mg/day on the second and subsequent days [11]. Furthermore, the use of favipiravir has been linked to toxic side effects in humans, including diarrhea, nephrotoxicity, elevated serum uric acid and transaminase levels, and reduced white blood cell and neutrophil counts alongside symptoms such as nausea, vomiting, abdominal pain, skin rash, itching, delirium, hallucinations, convulsions, and potential teratogenicity [12-14]. A recent experimental study revealed that favipiravir administration increased the levels of malondialdehyde (MDA), a toxic byproduct of lipid peroxidation (LPO), decreased the levels of the non-enzymatic endogenous antioxidant glutathione (GSH), and inhibited activities of enzymatic antioxidants such as superoxide dismutase (SOD), and catalase (CAT), in liver tissue [15]. Considering the documented side effects of favipiravir, its safety profile remains a concern based on a pooled analysis of extensive studies [9]. To the best of our knowledge, no literature studies have specifically investigated the impact of high-dose favipiravir on lung function.

Herein, we investigated the therapeutic effect of lacidipine, a drug derived from dihydropyridine and classified as an L-type calcium (Ca^{2+}) channel blocker, in mitigating the potential pulmonary toxicity caused by high-dose favipiravir [16]. Lacidipine has been primarily indicated for hypertension treatment [17]. Moreover, lacidipine exhibits antioxidant activity by inhibiting the increase in MDA levels and decrease in enzymatic and non-enzymatic antioxidant levels in organs and tissues [18]. In addition, lacidipine reportedly inhibits acute and chronic inflammation phases [19]. Thiamine pyrophosphate (TPP) is the active metabolite of thiamine, and we investigated the therapeutic effect of TPP against potential lung toxicity due to high-dose favipiravir [20]. Previous studies report that TPP exerts a protective effect by inhibiting elevated oxidant and proinflammatory parameters [21,22].

Moreover, we examined the therapeutic effect of adenosine triphosphate (ATP) against potential lung toxicity associated with high-dose favipiravir administration. ATP is a nucleoside triphosphate comprising adenine, ribose sugar, and three phosphate groups [23]. ATP also synthesizes reactive oxygen species (ROS)-scavenging antioxi-

dants[24]. In addition, ATP is an energy source for synthesizing low molecular weight antioxidants [25]. This study aimed to biochemically investigate and histopathologically evaluate the effects of lacidipine, TPP, and ATP on the possible oxidative lung damage caused by favipiravir in rats.

MATERIALS AND METHODS

Animals

In total, 36 albino Wistar-type male rats weighing 285–297 g were obtained from Erzincan Binali Yıldırım University Experimental Animal Research and Application Center. The experimental rats were subjected to standard environmental conditions, maintained at an ambient temperature of 22°C and a 12/12 h light–dark cycle. The rats had ad libitum access to animal feed and tap water. The study was approved by the local Animal Experiments Ethics Committee (Meeting Date: 25.08.2022; Meeting Number: 2022/08; Decision Number: 37).

Chemicals

Thiopental sodium was obtained from IE Ulagay (Turkey), favipiravir was obtained from the Ministry of Health Training and Research Hospital (Turkey), lacidipine was obtained from GlaxoSmithKline (Turkey), TPP was obtained from Biofarma (Russia), and ATP was obtained from Zdorove Narodu (Ukraine).

Experimental groups

The rats were classified into five groups: healthy (HG), favipiravir alone (Fav), lacidipine+favipiravir (LFav), TPP+favipiravir (TFav), and ATP+favipiravir (AFav).

Experimental procedure

Lacidipine (4 mg/kg orally), TPP (20 mg/kg IP), and ATP (25 mg/kg IP) were administered to the LFav (n = 6), TFav (n = 6), and AFav (n = 6) groups, respectively, to initiate the experiment. The HG (n = 6) and Fav (n = 6) groups were administered with distilled water. Following the 1-h interval following the administration of drugs and distilled water, favipiravir was orally administered at a dosage of 800 mg/kg twice daily for seven days to all the animal groups except the HG group. Favipiravir is known to cause oxidative and inflammatory damage at high doses [26]. Lacidipine, TPP, and ATP were administered once daily for seven days. TTP and ATP have been investigated before in these doses and found to be effective against oxidative stress [27,28]. Upon completion of this timeframe, the animals were euthanized via a high dose of thiopental sodium anesthesia (50 mg/kg), following which their lung tissues were extracted. MDA, tGSH, SOD, and CAT levels were measured in the excised lung tissues. Furthermore, the tissues were histopathologically examined. The biochemical and histopathological findings obtained from all the animal groups were compared and assessed for intergroup differences.

Biochemical analyzes

Preparation of samples

At this stage, 0.2 g of each removed tissue was weighed for biochemical examination. Tissue samples were washed with cold (+4°C) 0.15 M potassium chloride (KCl). Tissue samples were homogenized in liquid nitrogen. They were then passed into an ice-cold phosphate buffer solution (50 mM, pH 7.4). The tissue homogenates were centrifuged at 5000 rpm for 20 min at +4°C, and the supernatants were extracted to analyze MDA, tGSH, SOD, and CAT.

Determination of MDA, GSH, SOD, CAT, and protein

The levels of MDA, GSH, and SOD in the supernatants derived from the lung tissue samples from experimental animals were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (MDA catalogue no: 10009055; tGSH catalogue no: 703002; SOD catalogue no: 706002, Cayman Chemical Company).

CAT levels were determined according to the method proposed by Goth [29]. Protein content was determined spectrophotometrically at 595 nm according to the Bradford method [30].

Histopathological examination

The rats underwent necropsy for histopathological assessment, and their lung tissue samples were subsequently fixed in a 10% formalin solution. The tissues were subjected to a series of alcohol–xylene solutions and then embedded in paraffin blocks, from which 5-μm thick sections were obtained for histopathological evaluation. Then, these sections were stained using hematoxylin–eosin stain. The lung tissues were examined using a light microscope (Olympus BX51, Japan), and photographs were captured using a digital camera (Olympus DP 71) by a pathologist blinded to the treatment protocol. Histopathological damage in each tissue section was graded on a scale of 0–3 (0 = normal, 1 = mild, 2 = moderate, and 3 = severe) and assessed for mononuclear cell infiltrations in interstitial areas and lymphoid hyperplasia.

Statistical analysis

All statistical analyses for the biochemical findings of the experiment were conducted using IBM SPSS® Statistics for Windows, version 22.0 (IBM Corp, Armonk, NY, USA, released in 2013). A significance level of $p < 0.05$ was considered statistically significant. The biochemical results are expressed as mean ± standard error ($\bar{x} \pm$ SEM). The normality of distribution for continuous variables in the biochemical test results was assessed using the Shapiro–Wilk test. The significance level of the difference between the groups was determined using a one-way analysis of variance, as the distribution was normal. The Levene's test was performed to determine whether the homogeneity of variances was ensured. Following the assumption of homogeneity of variances, either the Tukey Honest Significant Differences test or the Games–Howell test was employed as a post hoc test. The histopathological findings were analyzed via the IBM SPSS® Statistics program for Windows®, version 20.0 (IBM Corp, Armonk, NY, USA, released in 2011). A nonparametric Kruskal–Wallis test was performed to assess between-group differences. Subsequently, the group responsible for the observed differences was identified using the Mann–Whitney U test at a significance level of $p < 0.05$.

RESULTS

Biochemical results

MDA analysis results of lung tissue

The lung tissue of the high-dose favipiravir–treated group exhibited higher MDA levels than those in the lung tissue of the HG, which was statistically significant (Table 1). Lacidipine, TPP, and ATP significantly suppressed the increase in MDA levels induced by high-dose favipiravir administration in lung tissue. However, ATP prevented the increase of MDA in lung tissue weaker than lacidipine and TPP. There was no significant difference in the MDA levels between the HG, lacidipine, and TPP groups.

tGSH analysis results of lung tissue

The lung tissue of the high-dose favipiravir–treated group exhibited lower tGSH levels than the lung tissue of the HG, and the difference was statistically significant (Table 1). Lacidipine and TTP significantly suppressed the decrease in tGSH levels induced by high-dose favipiravir administration in lung tissue. However, ATP administration did not exhibit a significant effect on preventing a decrease in tGSH levels. A statistically significant difference was detected in the tGSH levels between the lung tissue of the ATP-treated group and the HG. There was no significant difference in the tGSH levels between the HG, lacidipine, and TPP groups.

Table 1
Effect of lacidipine, TPP, and ATP on oxidant and antioxidant levels in lung tissue of rats administered with high doses of favipiravir.

Mean ± Standard error				
	MDA	tGSH	SOD	CAT
Groups	(nmol/mg protein)	(nmol/mg protein)	(U/mg protein)	(U/mg protein)
HG	1.65±0.07	6.63±0.08	9.55±0.05	8.51±0.06
Fav	5.80±0.06	2.56±0.05	3.56±0.06	3.53±0.09
LFav	1.82±0.03	6.31±0.15	9.19±0.15	8.14±0.12
TFav	2.72±0.34	5.34±0.34	6.49±0.34	6.18±0.36
AFav	3.84±0.23	2.79±0.19	3.84±0.11	3.66±0.06
Comparison of p-values				
Groups	MDA	tGSH	SOD	CAT
HG vs Fav	<0.001	<0.001	<0.001	<0.001
HG vs LFav	0.215	0.386	0.302	0.116
HG vs TFav	0.118	0.056	0.001	0.006
HG vs AFav	0.001	<0.001	<0.001	<0.001
Fav vs LFav	<0.001	<0.001	<0.001	<0.001
Fav vs TFav	0.001	0.002	0.001	0.003
Fav vs AFav	0.002	0.784	0.235	0.714
LFav vs TFav	0.201	0.163	0.001	0.011
LFav vs AFav	0.002	<0.001	<0.001	<0.001
TFav vs AFav	0.131	0.001	0.002	0.004

Abbreviations: TPP: thiamine pyrophosphate; ATP: adenosine triphosphate; HG: healthy group; Fav: alone favipiravir administered group; LFav: lacidipine + favipiravir group; TFav: TPP + favipiravir group; AFav: ATP + favipiravir group; MDA: malondialdehyde; tGSH: total glutathione; SOD: superoxide dismutase; CAT: catalase.

Footnotes: The Games-Howell test was applied as a post-hoc test after one-way ANOVA for all statistical evaluations.

SOD analysis results of lung tissue

The lung tissue of the high-dose favipiravir–treated group exhibited lower SOD activity compared with the lung tissue of the HG, and the difference was statistically significant (Table 1). Lacidipine and TPP significantly alleviated the decrease in SOD activity induced by high-dose favipiravir administration in lung tissue; however, ATP did not exhibit a similar effect. A statistically significant difference was found in the SOD activities between the lung tissue of the TPP and ATP groups and the HG. There was no significant difference observed in SOD activity between the HG and the lacidipine group.

CAT analysis results of lung tissue

CAT activity was lower in the lung tissue of the high-dose favipiravir–treated group than in the lung tissue of the HG, and the difference was statistically significant (Table 1). Lacidipine and TPP significantly suppressed the decrease in CAT activity induced by high-dose favipiravir administration in lung tissue; however, ATP did not suppress this decrease. A statistically significant difference was detected in the CAT activities between the lung tissue of the TPP and ATP groups and those of the HG. No significant difference was observed in CAT activity between the HG and the lacidipine group.

Histopathological results

Histopathologically significant differences were detected between the HG, Fav, LFav, TFav, and AFav groups (Table 2; $p< 0.05$). The lung tissue samples of the HG exhibited a normal histologic appearance (Fig. 1). Nevertheless, the rats in the high-dose favipiravir group exhibited severe mononuclear cell infiltrations in interstitial areas (Fig. 2A) and significant lymphoid hyperplasia in the lung tissue (Fig. 2B). In contrast, mononuclear cell infiltrations in interstitial areas (Fig. 2C) and lymphoid hyperplasia (Fig. 2D) were mild in the lacidipine group. In the TTP group, mononuclear cell infiltrations in interstitial areas (Fig. 3A) and lymphoid hyperplasia (Fig. 3B) were moderate. However, mononuclear cell infiltrations in interstitial areas (Fig. 3C) and lymphoid hyperplasia (Fig. 3D) were severe in the ATP group.

DISCUSSION

This study investigated the protective effects of lacidipine, TPP, and ATP against lung injury induced by high-dose favipiravir administration in rats. The investigation involved biochemical and histopathological analyses. Our biochemical experiments revealed that the MDA levels increased in the lung tissue of high-dose favipiravir–treated animals, whereas the tGSH, SOD, and CAT levels decreased significantly.

Our experimental results indicate that high doses of favipiravir may lead to severe oxidative damage. Herein, we measured MDA levels as it is a toxic byproduct of LPO and a significant indicator of oxidative stress [31]. A recent experimental study with results that align with our biochemical findings reported that favipiravir administration increased MDA levels in liver tissue. Additionally, the drug decreased endogenous antioxidant levels [15]. Similarly, Bilici et al. reported that rats treated with a high dose of favipiravir exhibited increased oxidant and decreased antioxidant levels [26].

Table 2
Effect of lacidipine, TPP, and ATP on histopathological scoring findings in lung tissue of rats administered with high doses of favipiravir.

Groups	MNC infiltrations in interstitial areas	Lymphoid hyperplasia
HG	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FAV	2.83 ± 0.40^{b}	2.83 ± 0.40^{b}
LFav	0.16 ± 0.00^{a}	0.16 ± 0.00^{a}
TFav	1.16 ± 0.40^{c}	1.16 ± 0.40^{c}
AFav	2.66 ± 0.81^{b}	2.56 ± 0.80^{b}

Abbreviations: TPP: thiamine pyrophosphate; ATP: adenosine triphosphate; MNC: mononuclear cell; HG: healthy group; Fav: alone favipiravir administered group; LFav: lacidipine + favipiravir group; TFav: TPP+ favipiravir group; AFav: ATP+ favipiravir group.

Footnotes: The values given are mean ± standard deviation values. a, b, c: Groups marked with the same letter are statistically similar, but there is a statistically significant difference at the level of $p < 0.05$ among groups with different letters.

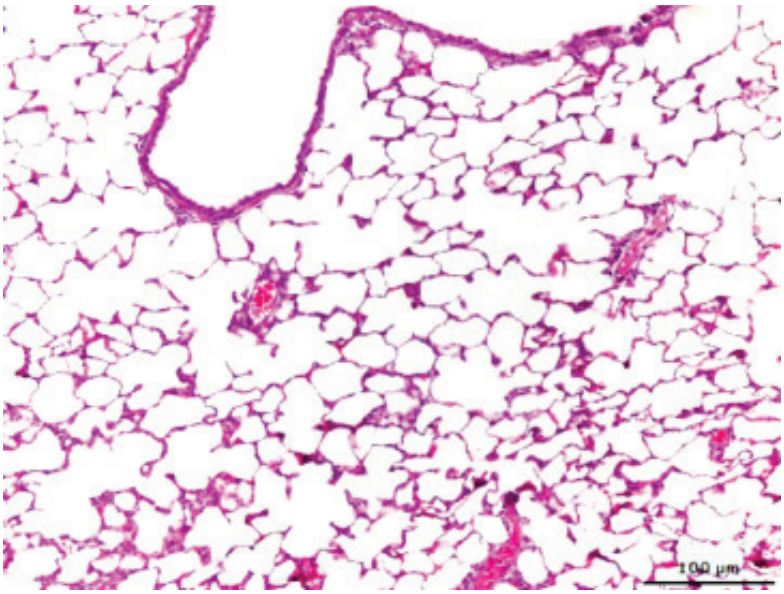

Fig. 1. Normal histological appearance of lung tissue belonging to the HG group (H&E x10).

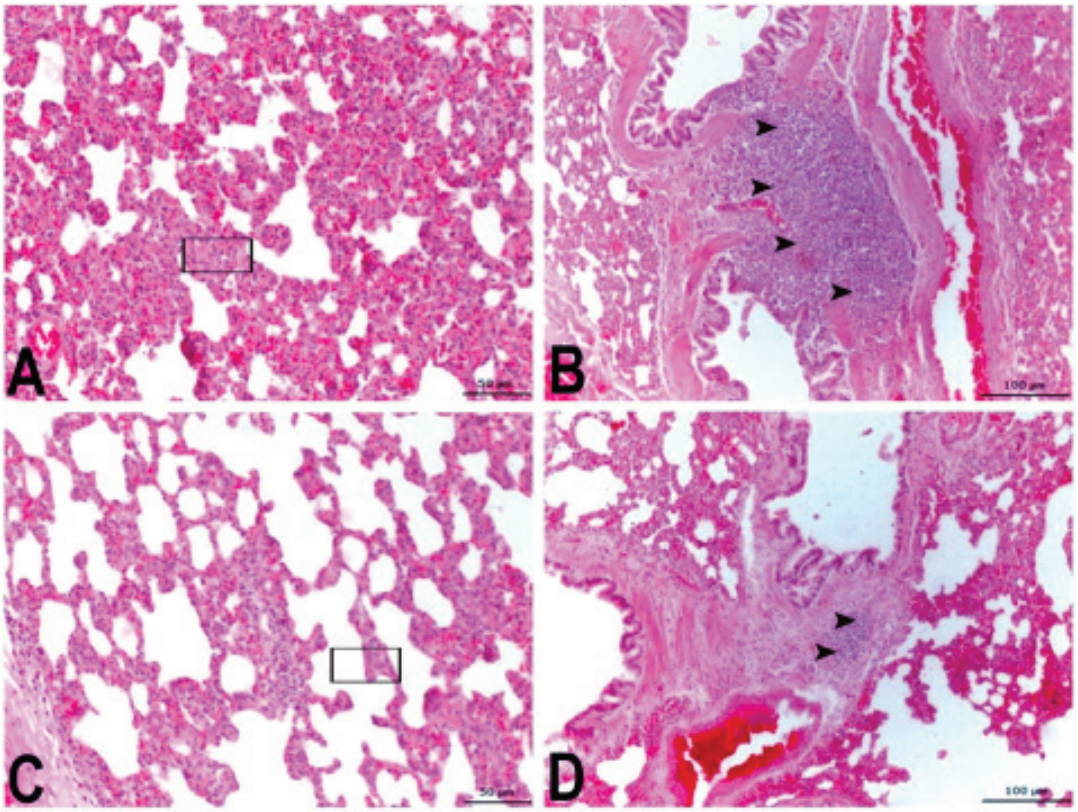

Fig. 2. (A) Severe mononuclear cell infiltrations in interstitial areas (□) appearance in the lung tissue belonging to the Fav group (H&E x20). (B) Severe lymphoid hyperplasia (arrowheads) appearance in the lung tissue belonging to the Fav group (H&E x10). (C) Mild mononuclear cell infiltrations in interstitial areas (□) appearance in the lung tissue belonging to the LFav group (H&E x20). (D) Mild lymphoid hyperplasia (arrowheads) appearance in the lung tissue belonging to the LFav group (H&E x10).

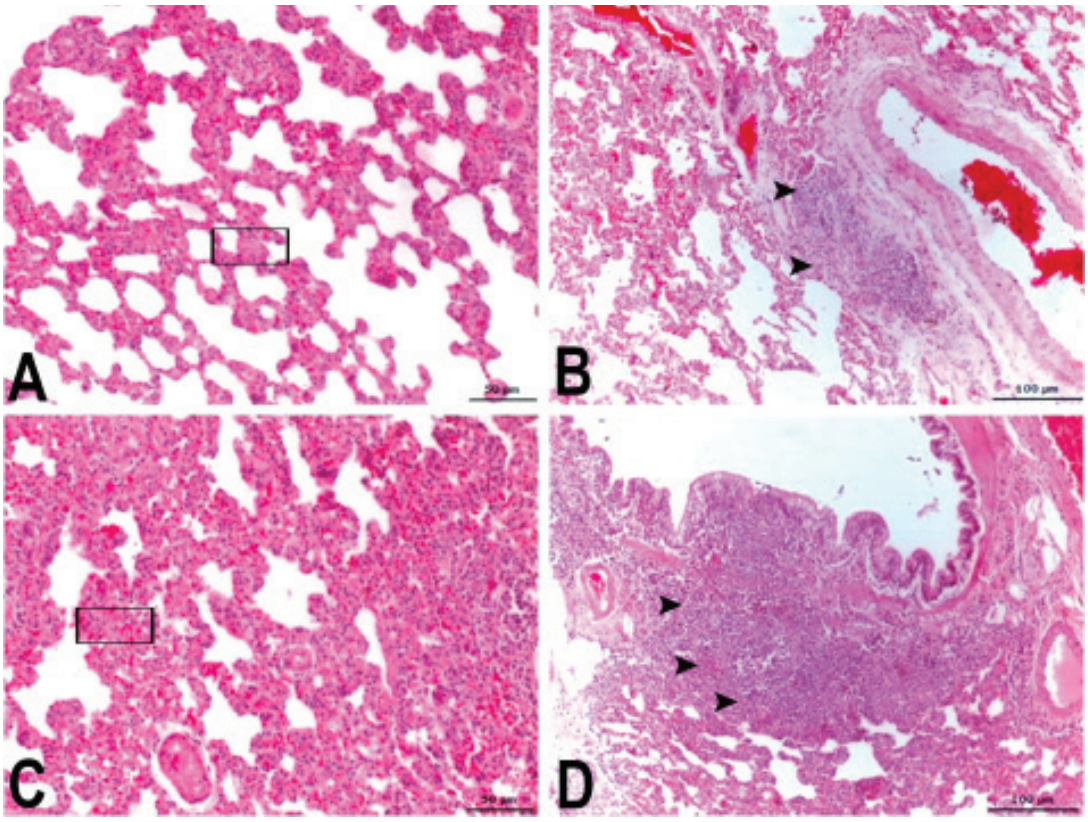

Fig. 3. (A) Moderate mononuclear cell infiltrations in interstitial areas (□) appearance in the lung tissue belonging to the TFav group (H&E x20). (B) Moderate lymphoid hyperplasia (arrowheads) appearance in the lung tissue belonging to the TFav group (H&E x10). (C) Severe mononuclear cell infiltrations in interstitial areas (□) appearance in the lung tissue belonging to the AFav group (H&E x20). (D) Severe lymphoid hyperplasia (arrowheads) appearance in the lung tissue belonging to the AFav group (H&E x10).

Conversely, lacidipine, TPP, and ATP administration significantly suppressed increased MDA levels in the studied subjects. In particular, it was observed that lacidipine suppressed the increase in MDA levels to a greater extent than TPP and ATP, bringing them closer to the levels of the healthy control group. To the best of our knowledge, there is no information in the literature regarding the effect of lacidipine, TPP, and ATP on lung injury induced by high-dose favipiravir. Previous studies have reported that lacidipine significantly decreased MDA levels and exerted a nephroprotective effect in cyclosporine-induced nephrotoxicity [32]. Previous research has also demonstrated that lacidipine exhibits the highest potency as an antioxidant among calcium channel antagonists, effectively inhibiting membrane LPO [33]. In addition, TPP exerts an antioxidant effect by significantly suppressing the cyclophosphamide-induced increase in MDA levels in female rats [21]. Similarly, a previous study reported that ATP protects kidney tissue from bevacizumab-induced oxidative damage by significantly inhibiting increased MDA levels [34].

In addition, enzymatic and non-enzymatic antioxidant parameters were measured to evaluate the possible oxidative damage of favipiravir in lung tissue. It is well-known that exposure to ROS from various sources activates a cascade of defense mechanisms in organs and tissues [35], such as endogenous antioxidants [36]. In case of insufficient antioxidant levels to counteract

the oxidant accumulation, oxidative stress occurs, leading to tissue damage [37,38]. As revealed by our experimental results, tGSH, SOD, and CAT levels decreased in lung tissue. It is widely recognized that GSH exists in two primary forms: the thiol-reduced form and the disulfide-oxidized form known as GSSG [39]. GSH has several functions, including antioxidant defense, detoxification, maintenance of thiol status, and modulation of cell proliferation [40]. The protective effect of GSH is attributed to its ability to react with ROS and effectively perform detoxification [41]. SODs are enzymatic antioxidants that play a role in the protection of cells against oxygen toxicity [42]. Likewise, CAT is a significant antioxidant enzyme in various cells that facilitates the breakdown of H_2O_2 into H_2O and O_2 [42]. Numerous prior studies have assessed the levels of oxidants and antioxidants above-mentioned to investigate the occurrence of lung damage [43].

Our study revealed that lacidipine and TPP administration effectively mitigated the decline in tGSH, SOD, and CAT levels. However, ATP did not demonstrate the same suppressive effect on these antioxidant markers. Moreover, lacidipine effectively reduced the decline in these antioxidant levels, restoring them to levels comparable with those observed in the HG. To the best of our knowledge, there is no data in the literature regarding the antioxidant effect of lacidipine against oxidative lung injury due to favipiravir. Several studies have reported that lacidipine protects heart tissue from oxidant damage [44]. In another study, lacidipine treatment reportedly attenuated the decrease in tGSH, SOD, and CAT levels [45]. It was determined that another drug, TPP, suppressed the decrease in tGSH levels and provided similar values as those of the healthy control group; however, there was a significant difference in SOD and CAT activities between the healthy control and TPP group. To the best of our knowledge, there was no data in the literature regarding the antioxidant effect of TTP against favipiravir-induced oxidative lung injury. In a previous study, TPP reportedly increased tGSH levels in oxidative optic nerve damage to levels comparable to those in the healthy group [46]. Demiryilmaz et al. reported that SOD and CAT activities increased in rats treated with TPP following oxidative liver damage [47]. Reportedly, ATP was ineffective in preventing the decline of enzymatic and non-enzymatic antioxidant levels and exhibited a noticeable difference compared to the values observed in the healthy control group. To the best of our knowledge, there were no reports in the literature investigating the antioxidant effect of ATP against favipiravir-induced oxidative lung injury. While Ozer et al. reported that ATP administration increased the enzymatic and non-enzymatic antioxidant levels in oxidative ovarian damage, our study did not yield similar findings [48].

Our study revealed a correlation between the biochemical results obtained from the lung tissues of the animals and histopathological findings. Severe mononuclear cell infiltrations and lymphoid hyperplasia in interstitial areas were observed in the lung tissue of the favipiravir group, wherein the oxidant levels were increased, and antioxidant levels were decreased. However, histopathological damage was observed to be alleviated in the lacidipine group, which best antagonized the effect of favipiravir on oxidant and antioxidant parameters. While histopathologic damage was moderate in the TPP group, which prevented oxidant increase and antioxidant decrease at a moderate level, severe histopathologic damage was detected in the ATP group, which could not significantly prevent the decrease of antioxidants caused by favipiravir. This is consistent with the literature reporting that histopathologic damage in lung tissue is associated with oxidant/antioxidant levels [49,50]. Pneumonia is a widely recognized condition characterized by inflammation of the lungs, typically due to an infection [51]. There is currently no information available in the litera-

ture suggesting that favipiravir induces interstitial inflammation via oxidative stress in the lungs. However, Tomoda Y *et al*. reported the development of drug-induced interstitial pneumonia in a patient receiving clopidogrel [52]. Jo T *et al.* reported that class III antiarrhythmic drugs, epidermal growth factor receptor inhibitors, and numerous other drugs can cause interstitial pneumonia [53]. Reportedly, the broncho-alveolar lavage fluid of a patient with drug-associated pneumonia contained abundant lymphocytes, macrophages, neutrophils, and eosinophils [52]. A study reported that ROS-induced oxidative stress may be one of the underlying factors in interstitial pneumonia development [54], supporting our biochemical and histopathological findings.

In conclusion, the systemic administration of high doses of favipiravir increased oxidant levels and decreased antioxidant levels in the lung tissue of rats. In parallel, the histopathological examination of the lung tissue revealed the presence of severe mononuclear cell infiltrations in interstitial areas and pronounced lymphoid hyperplasia. To the best of our knowledge, this was the first study focusing on the impact of high-dose systemic administration of favipiravir on lung tissue in terms of biochemical alterations and histopathological changes. Lacidipine exhibited superior efficacy in mitigating oxidative stress and preventing the decline of antioxidants induced by favipiravir compared with TPP and ATP. Histopathologically, lacidipine administration significantly reduced lung oxidative damage. TTP moderately reduced severe favipiravir-associated lung injury. However, ATP was ineffective against favipiravir-associated lung injury. Based on our findings, it can be concluded that lacidipine offers more therapeutic benefits than TPP in treating oxidative lung injury caused by high doses of favipiravir. We believe investigating pro-inflammatory and inflammatory cytokines can provide valuable insights into further understanding this issue.

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Conflict of interest

There are no conflicts of interest between the authors or between family members of the scientific and medical committees. The authors do not have any consultancy, expertise, working conditions, shareholdings or similar situations that could lead to potential conflicts of interest in any company.

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