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Research Article
Effect of Rutin on Pulmonary Injury in Everolimus-Administered Rats Biochemical and Histopathological Evaluation

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Abstract

Background and Objective: Everolimus is an anticancer agent that inhibits the Mammalian Target of Rapamycin (mTOR). It causes side effect such as pneumonia. This study aimed to evaluate rutin's effect on potential pulmonary injury in everolimus-administered rats biochemically and histopathologically. **Materials and Methods:** All rats were split into: Healthy (HG), everolimus-treated (EVR) (2 mg/kg) and rutin (50 mg/kg)+everolimus-treated (REV). Rutin was administered to the REV group and physiological saline was given orally tothe EVR and HG groups. As 1 hr later, everolimus 2 mg/kg was administered to REV and EVR groups. The process was repeated daily, 4 times weekly. Then, high dose anaesthesia was used to kill the rats and lung tissue samples were extracted. Oxidative stress and pro-inflammatory cytokine markers were examined and ANOVA test was applied. **Results:** Oxidative and inflammatory lung damage developed in the everolimus-treated rats. Rutin attenuated this oxidative and pro-inflammatory lung damage induced by everolimus. Rats administered with everolimus developed severe interstitial pneumonia, lymphoid hyperplasia and desquamation in lung tissues. This severe damage was reduced to mild level by administering rutin. **Conclusion:** The adding rutin to the treatment regimen for pulmonary damage associated with everolimus may have a preventive impact.

Key words: Everolimus, oxidative stress, rutin, pro-inflammatory, pulmonary damage, rat

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Everolimus is an anticancer agent that inhibits the Mammalian Target of Rapamycin (mTOR)[1]. After being confirmation by the Food and Drug Administration in 2011[2], it has been preferred in many countries for treating breast cancer, as well as in liver, kidney and lung transplants, as well as stents[3-7]. The positive effects of everolimus in treatment are accompanied by its side effects. After ingestion, it is well tolerated by the body; however, it may cause side effects such as anemia, asthenia, fatigue, stomatitis, pneumonia, leukopenia and hyperglycemia[8]. Like other mTOR inhibitors, everolimus also leads to certain organ toxicities in clinical applications[9]. Pneumonitis has become a life-threatening complication in the clinical use of everolimus in recent years[10]. Everolimus induces pneumonia by stimulating proinflammatory cytokines in monocytes and macrophages[11]. Cases of alveolar hemorrhage and interstitial pneumonia have been reported in patients using everolimus in the literature[12,13]. In another study informed that everolimus exacerbates oxidative stress by disrupting the redox balance in tacrolimus-induced organ damage[14]. Additionally, oxidative stress leads to a rise in proinflammatory cytokine levels, such as TNF-α and interleukin-6 in the tissue[15]. So, this method may be useful in treating everolimus-induced pulmonary toxicity.

Rutin, a flavonoid also known as vitamin P, which could have a preventive impact against potential pulmonary damage in animals treated with everolimus, possesses antioxidant, anti-inflammatory, antiallergic, antiviral and anticarcinogenic properties[16]. It is predominantly found in onions, red wine, apples and tea[17]. Studies have shown that rutin reduces oxidative damage associated with increased reactive oxygen species (ROS)[18]. Tosun *et al.*[19] was presented that rutin exhibited antioxidant and anti-inflammatory effects in the lung tissues of rats. In another study, rutin was shown to reduce alveolar edema and inflammation induced by methotrexate in lung tissues[20]. However, there are no research exploring the effect of rutin against potential pulmonary injury caused by everolimus. Therefore, this research aimed to evaluate the impact of rutin on everolimus-induced lung injury in rats, both biochemically and histopathologically.

MATERIALS AND METHODS

Study area: This study was carried out Ataturk University, Faculty of Medicine, Erzurum/Türkiye from July to August, 2020.

Animals: In the experiment, eighteen albino Wistar male rats with weights ranging from 262 to 277 g were used. Whole experimental animals used in the study of Voutsadakis[1] were obtained from the Ataturk University Medical Experimental Application and Research Center. During the experiment, the room temperature was kept at normal levels (22°C) and fed for 1 week to allow them to acclimatize to their surroundings.

Ethical consideration: All stages of the study were carried out ethical principles and accepted by the Ataturk University Local Ethics Committee for Animal Experiments on May 29, 2020 (Decision No: 5).

Chemical substances: Everolimus was supplied from Novartis (Türkiye), Thiopental Sodium I.E. Ulagay (Türkiye) and Rutin Solgar (USA).

Experimental groups: For the experiment, rats were separating into: Healthy (HG), everolimus-treated (EVR) (2 mg/kg) and rutin (50 mg/kg)+everolimus-treated group (REV).

Experimental procedure: In the REV group of rats (n = 6), the rutin was given via oral gavage at 50 mg/kg dose. The EVR (n = 6) and HG (n = 6) groups were given physiological saline solution (0.9% NaCl) via oral gavage at the same volume as the solvent. An hour later, administering rutin and 0.9% NaCl, everolimus was administered to the REV and EVR groups via gastric gavage at a dose of 2 mg/kg once daily repeated for four weeks. At the end of this period, all rats were euthanized high-dosage anesthesia (50 mg/kg thiopental sodium) and then lung tissue samples were taken. The extracted lung tissues were examined both biochemically and histopathologically. All findings taken HG and EVR groups were compared with the REV group.

Biochemical analysis: Tissue samples were first cleaned with a 0.9% NaCl solution and then put into a Petri dish. All samples were ground into powder using liquid nitrogen. The MDA, GSH, NF-κB and TNF-α protein levels were assayed by homogenisation of the tissue samples.

MDA, tGSH, NF-κB and TNF-α measurement: The MDA was identified using the method of Ohkava *et al.*[21], tGSH was determined using the method of Sedlak and Lindsay[22]. An Enzyme-Linked Immunosorbent Assay (ELISA) kit for experimental animals was used to measure the levels of NF-κB and TNF-α supernatants prepared from lung tissue samples

(Cat No: 201-11-0288 SunRed and Cat No: YHB1098Ra, Shanghai LZ). The manufacturer's instructions were followed for analysis.

Histopathological examination: Lung tissues were fixed in 10% neutral formalin. Tissues were submitted to a standard alcohol-xylene series and processed through paraffin embedding. The 4µ sections were coloured with haematoxylin-eosin and graded semi quantitatively as none (-), mild (+), medium (++) and severe (+++) with respect to interstitial pneumonia, lymphoid hyperplasia and desquamation of bronchial-bronchiolar epithelial cells. Photographs were taken after evaluation using the Olympus DP2-SAL firmware program (Olympus® Inc. Tokyo, Japan).

Statistical analysis: The findings were shown as "Mean±Standard Deviation" ($\bar{\chi}\pm$SD). All biochemical analyzes were performed by one-way Analysis of Variance (ANOVA) and used Tukey's t-test for pairwise comparison between groups. All statistical procedures were performed using the "IBM Statistical Package for the Social Sciences Statistics (SPSS) 25.0" program, figures were designed in "GraphPad Prism 9" in addition $p<0.05$ had statistical significance.

RESULTS

Biochemical results

MDA and tGSH analyses: As seen in Fig. 1(a-b), rats administered with everolimus exhibited increased MDA levels

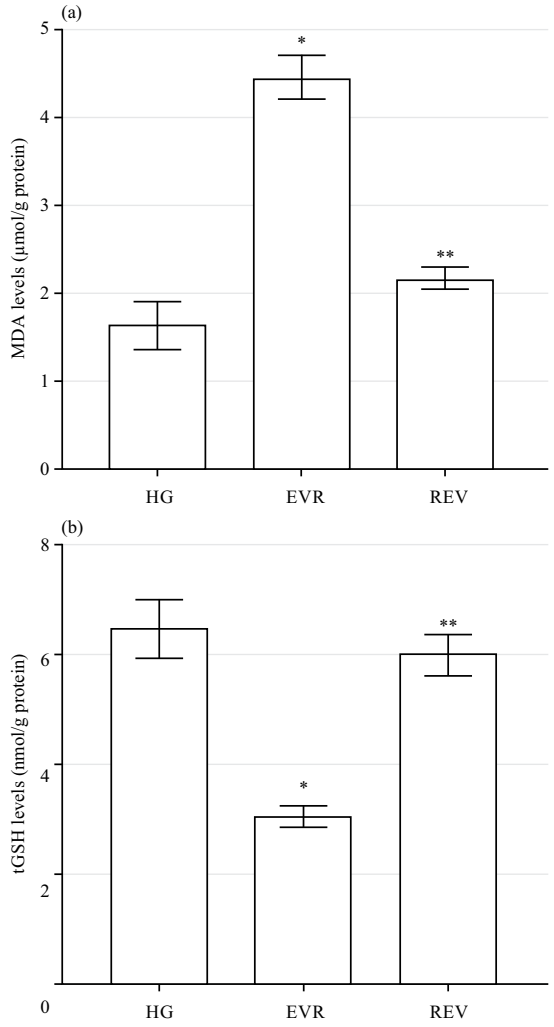

Fig. 1(a-b): (a) MDA and (b) tGSH levels in lung tissues of study groups

MDA: Malondialdehyde, tGSH: Total glutathione, HG: Healthy, EVR: Everolimus-treated, REV: Rutin+everolimus treated group, *p<0.001 vs HG and **p<0.001 vs EVR group (n-6)

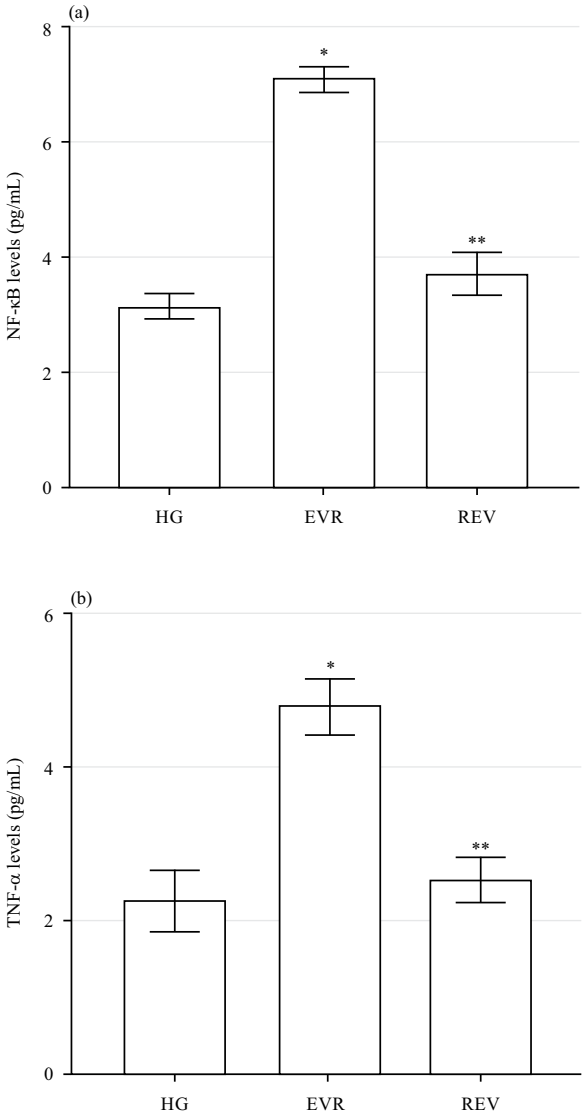

Fig. 2(a-b): NF-κB and TNF-α levels in lung tissues of study groups, (a) NF-κB: Nuclear Factor-Kappa and (b) TNF-α: Tumor Necrosis Factor α

HG: Healthy, EVR: Everolimus-Treated, REV: Rutin+everolimus treated group *p<0.001 vs HG and **p<0.001 vs EVR group (n-6)

and decreased tGSH levels in lung tissues vs the HG (p = 0.002). In the REV group, rutin significantly inhibited the increase in MDA levels and the decrease in tGSH levels induced by everolimus (p = 0.002). No statistically significant difference between the REV and healthy groups was found in MDA and tGSH levels (p = 0.133).

NF-κB and TNF-α analyses: As seen in Fig. 2(a-b), NF-κB and TNF-α levels were increased in the lung tissues of the rats in the EVR group vs the HG (p<0.001). In the REV group administered with rutin, the increased NF-κB levels decreased vs the healthy group (p = 0.009). No statistically significant difference was found in TNF-α levels between the REV and healthy groups (p = 0.384) (Table 1).

Histopathological findings: In the HG, the lungs of rats exhibited a normal histological appearance (Fig. 3).

In the EVR group administered with everolimus, severe interstitial pneumonia, lymphoid hyperplasia and moderate desquamation in the bronchiolar epithelium were observed Fig. 4(a-b).

In the REV group administered with rutin, mild interstitial pneumonia, lymphoid hyperplasia and desquamation in the bronchiolar epithelium were observed Fig. 5(a-b).

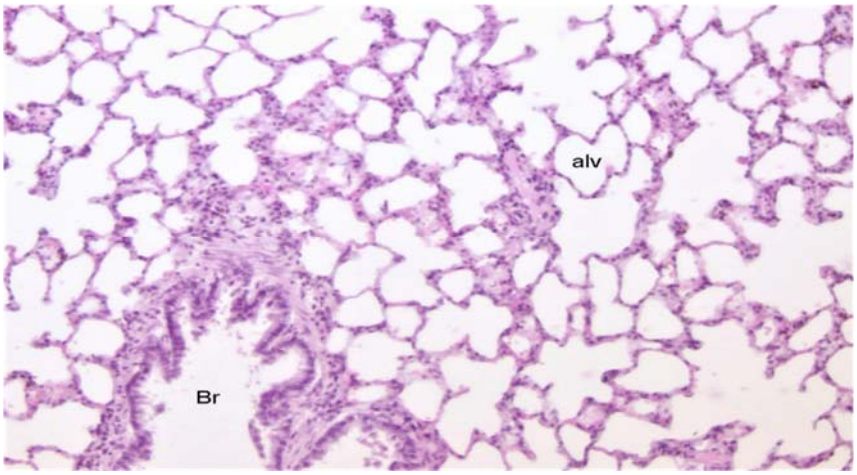

Fig. 3: Normal lung histopathological appearance
Br: Bronchiole, alv: Alveoli and H&E×20

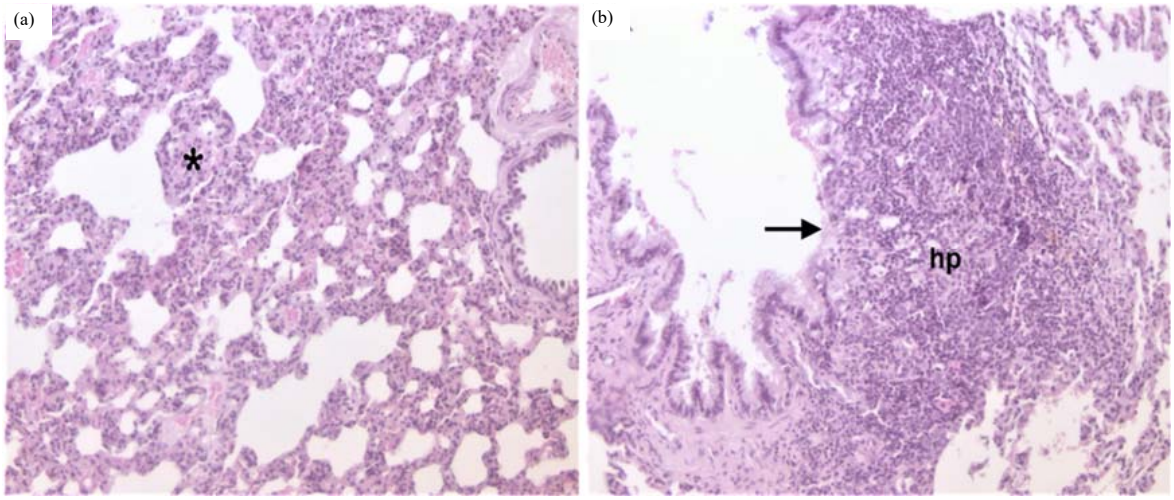

Fig. 4(a-b): Rat lung tissue belonging to the EVR group
EVR: Everolimus-treated group, *Severe interstitial pneumonia, lymphoid, hp: Hyperplasia, moderate desquamation of the bronchiolar epithelium (arrow) and H&E×20

Table 1: Analysis of biochemical parameters in lung tissue of study groups

	Mean±Standard Deviation median (Min-Max)			p-value		
Biochemical parameter	HG	EVR	REV	HG-EVR	HG-REV	EVR-REV
MDA (µmol/g protein)	1.65±0.27	4.47±0.25	2.19±0.12			
	1.7 (1.25-1.92)	4.4 (4.14-4.78)	2.15 (2.11-2.41)	0.001	0.002	0.001
tGSH (nmol/g protein)	6.49±0.53	3.08±1.88	6.02±0.37			
	6.58 (5,55-6.99)	3.11 (2.79-3.36)	6.16 (5.3-6.33)	0.001	0.133	0.001
NF-κB (pg/mL)	3.15±0.20	7.07±0.22	3.70±0.38			
	3.15 (2,,90-3.41)	7.12 (6.75-7.31)	3.69 (3.12-4.21)	0.001	0.009	0.001
TNF-α (pg/mL)	2.25±0.38	4.78±0.38	2.53±0.29			
	2.17 (1.96-3.00)	4.81 (4.14-5.14)	2.48 (2.22-2.98)	0.001	0.384	0.001

MDA: Malondialdehyde, tGSH: Total glutathione, NF-κB: Nuclear factor-kappa B, TNF-α: Tumor necrosis factor HG: Healthy, EVR: Everolimus-treated and REV: Rutin+everolimus treated group. All analyzes were performed by One-way Analysis of Variance (ANOVA) and the Tukey's t-test was used for pairwise comparison between groups (N = 6) $p<0.05$ was considered significant

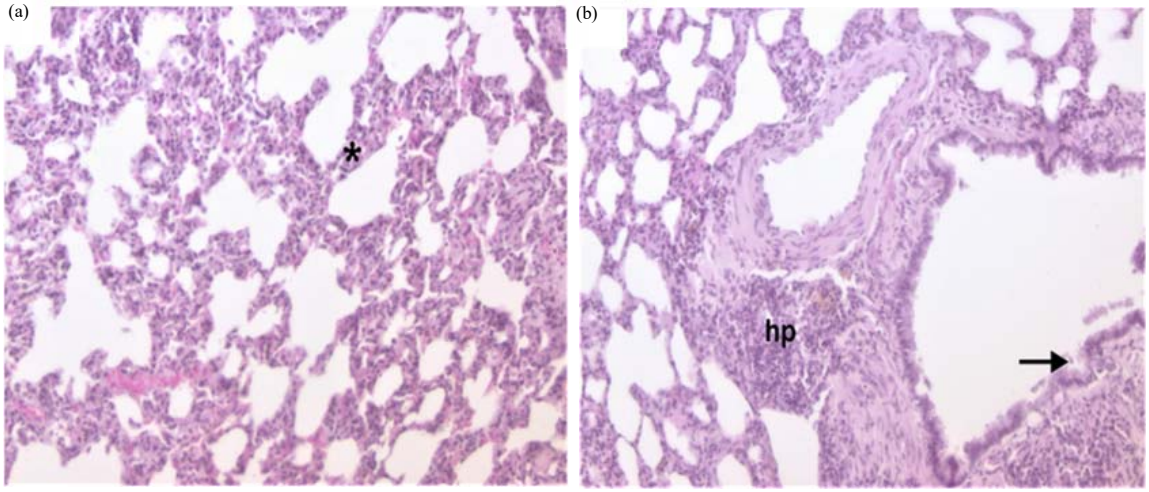

Fig. 5(a-b): Rat lung tissue belonging to the REV group

REV: Rutin+everolimus treated group *Mild interstitial pneumonia, lymphoid, hp: Hyperplasia desquamation of bronchiolar epithelium (arrow) and H&E×20

DISCUSSION

In this research, the effect of rutin on potential lung damage associated with everolimus use was examined biochemically and histopathologically. Everolimus is known as an mTOR inhibitor and anticancer agent[1]. Additionally, it is known to be indicated in various cancer treatments and organ transplantations[3-7]. It is also used in long-term cancer care. One of its most significant side impacts is pneumonia, occurring in approximately 10% of cases[8]. Literature reviews have reported cases of interstitial pneumonia, interstitial lung toxicity and oral toxicity associated with the use of everolimus[23-25]. The findings obtained in the present study indicated that everolimus leads to an increase in MDA levels in lung tissues, while rutin inhibits the increase in MDA levels. In organisms, oxidative damage occurs due to the imbalance between oxidant and antioxidant systems, associated with increased ROS levels[26]. The MDA is a popular and reliable indicator of oxidation damage[27]. Piao *et al.*[14] showed that everolimus exacerbated oxidative damage by causing an increase in MDA levels. Tosun *et al.*[19] reported that rutin normalized MDA levels in lung tissue, thereby reducing the severity of oxidative damage.

Oxidative damage occurs when the oxidant/antioxidant balance shifts towards the oxidants. In all aerobic organisms, both physiological and pathological processes generate ROS[28]. One of the antioxidants that neutralize the increased ROS associated with oxidative damage is GSH[29]. Everolimus treatment decreased tGSH levels in the lung tissue of rats in this study, while rutin inhibited the decrease in tGSH, bringing it closer to the level in healthy tissue. Topal *et al.*[18]. reported that rutin reduced MDA levels and restored GSH levels to normal, significantly suppressing oxidative damage induced by cisplatin-induced cardiac injury. Additionally, literature reviews have reported that rutin reduces increased MDA levels while increasing decreased GSH levels in cisplatin-induced jejunal and vestibular nerve damage, as well as in immobilization-induced stress-related heart damage[30,31]. Everolimus has been used in various cancer treatments for years due to its anticancer effects. As known, NF-κB pathways are activated in various cancer types and are generally associated with poor prognosis[32]. Studies in the literature report that everolimus inhibits the production of NF-κB[33,34]. However, an *in vivo* study reported that levels increased after renal ischemia-reperfusion in rats administered with everolimus[34]. As the experimental results of the present study show, NF-κB levels in the lung tissues of everolimus-administered rats increased compared to the healthy group. In contrast, NF-κB levels in REV were very close to those in HG. It is known that rutin has an inhibitory effect on NF-κB levels[35]. Rutin effectively reduced the increased NF-κB levels induced by everolimus, the study results showed.

Another cytokine that plays a role in inflammation is TNF-α. It is usually released by macrophages and monocytes[36]. The TNF-α levels in lung tissues of rats administered with

everolimus were higher versus the HG. However, in the group administered with rutin, the increased TNF-α levels were inhibited, approaching those of the HG. A Wo *et al.*[37] reported that the TNF-α levels induced by cisplatin significantly decreased when administered with low, medium and high doses of rutin.

Biochemical findings in this study agree with histopathological's. Animals administered with everolimus developed severe interstitial pneumonia, lymphoid hyperplasia and desquamation in lung tissues. This severe damage was reduced to a mild level by administering rutin. There are also studies reporting that everolimus induces interstitial pneumonia[38,39]. Bai *et al.*[40] investigating the effects of rutin on interstitial pneumonia, lymphoid hyperplasia and desquamation have not been published in the literature. However, one study reported the positive impact of rutin in bleomycin-induced lung fibrosis[40].

CONCLUSION

This is the first and only study in the literature to investigate the effects of rutin on everolimus-induced lung injury. The biochemical and histopathological results obtained in this study showed that oxidative and inflammatory damage developed in the lung tissue of everolimus-treated rats. Rutin attenuated this oxidative and pro-inflammatory lung damage induced by everolimus. These results suggested that rutin may be benefical in the care of everolimus-induced pulmonary toxicity.

SIGNIFICANCE STATEMENT

This study found that routine ameliorated everolimus-induced oxidative damage in rat lung tissues. Rutin minimized tissue damage by inhibiting the increase in MDA, NF-κB and TNF-α levels and the suppression of tGSH level in lung tissues. Effective dose ranges of rutin against everolimus toxicity could not be determined. Comparative studies between rutin and currently available antioxidants and anti-inflammatory drugs should be conducted to evaluate the protective effect of rutin against everolimus-induced pulmonary toxicity. Such investigations may contribute to understanding the potential of rutin about everolimus.

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