# A New Approach to Sepsis Treatment by Rasagiline: A Molecular, Biochemical and Histopathological Study

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# Abstract

**Aim:**We aimed to investigate the effects of rasagiline, which has a strong antioxidant, anti-apoptotic and anti-inflammatory effect, on acute lung injury that develops in the sepsis model induced with the CLP in rats.

**Main Methods:**The rats were separated into the following six groups, Group 1: Sham, Group 2: Sham + Rasegiline 4 mg/kg, Group 3: Sepsis, Group 4: Sepsis + Rasegiline 1 mg/kg, Group 5: Sepsis + Rasegiline 2 mg/kg, Group 6: Sepsis + Rasegiline 4 mg/kg. A total of 4 holes were opened with a 16-gauge needle through the cecum distal to the point of ligation.

**Key Findings:**GSH levels appear to improve due to increased doses of rasagiline, while SOD activity appears to improve only at the high dose of rasagiline. There was a statistically significant improvement in the doses of R2 and R4. This improvement in Tnf-α, IL1β, IL6, NF-κβand HMGB1 expression increased dose-dependent at R2 and R4 doses. In increased doses, rasagiline appears to prevent the development of edema, the formation of inflammation, and hemorrhagic areas are almost similar to healthy tissue.

**Significance:** Rasagiline exerts both antioxidant and anti-inflammatory effects on CLP induced acute lung injury in rats.


# Introduction

Sepsis is a life-threatening organ dysfunction caused by the uncontrolled immune response of the host against the infection.[1]There are still delays in diagnosing of sepsis and associated conditions although it is associated with high morbidity and mortality. Together with the complexity of the sepsis pathogenesis, it is well known that immune cells such as neutrophils, monocytes and macrophages play the leading role.[2]

Released proinflammatory mediators and cytokines create complement pathway activation and neuroendocrine response.[3]Proinflammatory cytokines play a fundamental role in the sepsis diagram,especially in the first 24 hours, and initiate the organ failure.[4]The effects of host defense cells begin with their recognition of microbial components such as binding of toll-like receptor-2 (TLR-2) to gram positive bacterial peptidoglycan.Cytosolic nuclear factor-kappa beta (NF-κβ) signal cascade is activated and then moves from the cytoplasm to the nucleus. NF-κβbinds to transcription sites of the nucleus and induces proinflammatory cytokines as tumor necrosis factor alpha (Tnf-α) and interleukin-1 beta(IL1β), chemokines as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and the gene cluster responsible for nitric oxide release.[5]Polymorphonuclear leukocytes (PNL) are activated and migrates to the scene in a series of steps (rolling, adhesion, diapedesis, chemotaxis) with the help of mediators released from the endothelium.[6]Release of mediators by PNLs in the infection area causes local infection cardinal symptoms such as local vasodilation, hyperemia, and temperature increase.

Treatment in sepsis usually begins with the patient's admission to the emergency room and continues under hospital service or intense care unit conditions. Basically, treatment can be divided into drug therapy and supportive therapy. Antimicrobial, antiviral, antifungal and vasopressor agents, inotropes and corticosteroids are used in drug treatment.[7]Support therapy mainly includes fluid resuscitation, respiratory support, source control, blood product support, glycemic control, deep vein thrombosis prophylaxis, stress ulcer prophylaxis, renal replacement therapy and nutritional therapy. Many treatment modalities have been investigated for the control and treatment of inflammation and accompanying physiological disorders seen in acute lung injury. Corticocyteroids, neutrophil elastase inhibitor, granulocyte-macrophage colony stimulating factor, statins, omega-3 fatty acids are some of the anti-inflammatory treatments that have been studied against the acute lung injury. Although its effects have been demonstrated in experimental models, the search for effective treatment continues, as a clinically significant effect on human mortality has not been proven.

Rasagiline, an irreversible MAO-B inhibitor, used to treat Parkinson's disease.It prevents the breakdown of dopamine by inhibiting MAO-B.[8] In many diseases, including Parkinson's disease, rasagiline has been shown to have an anti-apoptotic effect and a neuroprotective effect.[9, 10]Although the neuroprotective effect of rasagiline is thought to be due to MAO-B inhibition, there are studies showing that this effect is independent from MAO-B inhibition.[11, 12] In a study conducted by Kranthi et al., it was shown that Rasagiline has a strong antioxidant effect.It has also been shown that Rasagiline protects cell survival by inhibiting mitochondrial membrane permeability, which initiates cell death.[13]Varela et al. demonstrated the cardioprotective effects of rasagiline in the experimental myocardial infarction model.[14]In this case, it is thought that rasagiline may have effects on peripheral diseases besides its effects on central diseases.

In a previous study related with our subject, it was shown that rasagiline exhibited anti-inflammatory effect by preventing the increased IL1βrelease due to inflammation induced by lipopolysaccharide in vitro.[15] It was also shown in this study that rasagiline can suppress ROS production and prevent mitochondrial membrane potential. However, there has been no previous study on the effect of rasagiline on sepsis.Therefore, in our study, we aimed to investigate the effects of rasagiline, which has a strong antioxidant, anti-apoptotic and anti-inflammatory effect, on acute lung injury that develops in the sepsis model induced with the cecal ligation and puncture (CLP) in rats.

## Material And Methods

## Animals

Totally48female Albino Wistar rats at age 10 to 12 weeks old weighing 220 to 240 g was used in the experiment. This study was approved by and performed in accordance with the institutional animal care and use ethics committee of Ataturk University with the protocol number 2019-6/98. All animals were housed under the proper light and temperature conditions by giving regular feed and tap water during the experiment.[16]

# Experimental Design

The rats were separated into the following six groups, and each group consisted of eight rats:

Group 1: Sham (Surgical Procedure)

Group 2: Sham + R4 (Rasegiline 4 mg/kg)

Group 3: Sepsis (Sepsis with Cecum Ligation and Puncture model)

Group 4: Sepsis + R1 (Rasegiline 1 mg/kg)

Group 5: Sepsis + R2 (Rasegiline 2 mg/kg)

Group 6: Sepsis + R4 (Rasegiline 4 mg/kg)

# Cecal ligation and puncture sepsis model

Animals were fasted for 12 h before surgery with free access to water. Rasagiline was administered orally at three doses: 1 mg/kg,2 mg/kg and 4 mg/kg dissolved in saline.[17]Sepsis was applied one hour after rasagiline application with the CLP model.[18]The CLP sepsis model was appliedto the rats anesthetized by intraperitoneal administration of thiopental at 20 mg/kg and inhaled 5 % sevoflurane. After anesthesia, abdomens were shaved and 2 cm diameter incision was made in the abdominal midline of each rat, and the cecum was isolated and tied with a 3/0 silk ligature just distal to the ileocecal valve.A total of 4 holes were opened with a 16-gauge needle through the cecum distal to the point of ligation. After this step the cecum was replaced to the peritoneal cavity. The abdominal incision was then closed with a 4/0 sterile synthetic absorbable suture in two layers. In the Sham groups, the cecum was manipulated but not ligated or perforated. Post anesthetic analgesia was achieved with an intramuscular administration of metamizole sodium. All animals subcutaneously received 2 ml of normal saline intraoperatively and 6 hours postoperatively for fluid resuscitation. The rats were deprived of food postoperatively but had free access to water for the next 16 h until they were sacrificed.[19, 20]

The experiment was terminated 16 h after sepsis was created. All groups were sacrificed with an overdose of a general anesthetic (thiopental sodium, 50 mg/kg), and whole blood samples were withdrawn via the intracardiac method.The lung tissues were removed and divided into two parts. Half of the lung tissues were fixed in 10%formalin for histopathological analyses and the other half were stored at − 80°C for biochemical and molecular analyses.

# Biochemical Analysis

Lung tissues were fixed with liquid nitrogen and 50 mg pulverized tissue homogenized with TissueLyser II (Qiagen) in 1 mL PBS solution. Supernatant was used as sample for the measure of the superoxide dismutase activity (SOD)[21], glutathione (GSH)[22] and malondialdehyde levels (MDA)[23]according to

the modified methods with multiwell plate reader.[24]Total protein levels were analyzed by the Lowry Method, using commercial protein standards (SigmaAldrich, TP0300).

# Gene Expressions Analyses

Lung tissues (30 mg) were fixed with RNA stabilization reagent (RNAlater, Qiagen). Then, liquid nitrogen was used to freeze tissues and Tissue Lyser II was used to homogenize the samples. Total RNA was isolated by using RNeasy Mini Kit Qiagen according to the instructions of the manufacturer with QIAqube (Qiagen). The RNA samples were reverse-transcribed into complementary DNA using a high-capacity cDNA reverse transcription kit (Applied Biosystem). The cDNA concentrations were measured and quantified using the Epoch Spectrophotometer System and Take3 Plate (Biotek).[25]

Tnf-α, IL1β, IL6, NF-κβ, HMGB1 and NLRP3 expression analyses were performed with Step One Plus Real Time PCR System technology (Applied Biosystem). Real-time PCR was performed using primers designed for rat Tnf-α Rn99999017_m1, rat IL1β Rn00580432_m1, rat IL6 Rn01410330_m1, rat NF-κβ Rn01399583_m1, rat HMGB1 Rn02377062_g1 and rat NLRP3 Rn04244625_m1 and rat β-actin Rn00667869_m1. Beta-actin was used as endogenous controls. For each group, triplicate determinations were performed in a 96-well optical PCR plate and all quantification of gene expression steps were performed as described previously. All data were expressed as fold change in expression compared to the control (sham) group using the $2^{-\Delta\Delta Ct}$ method.[26]

# Histological analyses

Lung tissues in all groups were taken and fixed in 10% neutral formalin solution. Then, routine pathological processing was done and lung tissues were embedded in paraffin wax, and 4–5 μm thick serial sections were cut. All tissue sections were stained with hematoxylin and eosin for histopathology assessment before being examined under a light microscope (Olympus BX51, Japan). For histopathological assessment, edema, inflammation, bleeding and hemorrhagic areaswere evaluated for lung tissues.[27]In the present studydifferent areas were examined randomly in each lung and scoring tables were created. A minimum of five fields for each tissue slide at ×100 magnification were evaluated and assigned to determine the severity of the changes using scores on a scale where Grade 0: - (0% negative), Grade 1: + (0–33% mild positive), Grade 2: ++ (33–66% moderate positive), Grade 3: +++ (66–100% severe positive).[28]

# Statistical analysis

SPSS 20.0 softwarewas used for the statistical analyze and the results were presented as means ± standard deviation (S.D.). Statistical analysis of molecular results (Tnfα, IL1β, IL6, NF-κβ, HMGB1 and NLRP3) and biochemical results (SOD,GSH and MDA) were performed using one-way ANOVA and the Tukey multiple comparison test. Significant differences were detected between all groups, compared to the Sham group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$), compared to the Sepsis group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$) and compared to the Sepsis + R4 group (δ $P < 0.05$, δδ $P < 0.01$, δδδ $P < 0.001$).

## Results

# Antioxidant and Oxidative stress findings

It was shown that SOD and GSH levels significantly decreased due to oxidative damage in the sepsis group's lungs in the CLP-induced sepsis model (Fig. 1A-B). In rasagiline groups, oxidative stress is observed to improve in dose dependent manner .Compared to the control group, GSH levels appear to improve due to increased doses of rasagiline, while SOD activity appears to improve only at the high dose of rasagiline. Rasagiline did not show any effects on the oxidative stress in alone administered groups.

In the present study, in the model of sepsis created with CLP, MDA levels significantly increased in the lungs of the sepsis control group due to oxidative damage compared to the Sham group (Fig. 1C). It was observed that oxidative stress decreases depending on dose in rasagiline groups. Compared to the sepsis control group, MDA levels appear to decrease due to increased doses of rasagiline.

# Inflammatory findings

In the present study, looking at the Tnf-α, IL1β, IL6, NF-κβ, HMGB1 and NLRP3 mRNA expressions in the lung tissues of sepsis group, a statistically significant increase was observed compared to the sham group (Fig. 2A-F).In the Rasegiline administered groups, there was no significant improvement in the dose of R1, while there was a statistically significant improvement in the doses of R2 and R4. This improvement in Tnf-α, IL1β, IL6, NF-κβ and HMGB1 expression increased dose-dependent at R2 and R4 doses, while there was no significant difference between them in HMGB1 expression (Fig. 2A-F).

# Histopathological findings

Pathological scores were shown in Table 1. Although no pathological signs were found in the lung tissues of the Sham group (Fig. 3A), moderate edema, moderate inflammation, moderate bleeding were found in the lung tissues of the sepsis group, and severe vascular congestion was detected (Fig. 3B). In rasagiline groups, low dose administration does not show any protective effect on sepsis (Fig. 3C). In increased doses, rasagiline appears to prevent the development of edema, the formation of inflammation, and hemorrhagic areas are almost similar to healthy tissue (Fig. 3D-F).

Table 1
Effects of rasagiline treatments on histopathological scores of the lung tissues

| Groups | Edema | Inflammation | Hemorrhage | Vascular congestion |
|---|---|---|---|---|
| Sham | - | - | - | - |
| Sepsis | +++ | +++ | +++ | +++ |
| Sepsis + R1 | ++ | ++ | ++ | +++ |
| Sepsis + R2 | + | + | - | - |
| Sepsis + R4 | - | - | - | - |
| Sham + R4 | - | - | - | - |

R1: 1 mg/kg Rasagiline, R2: 2 mg/kg Rasagiline, R4: 4 mg/kg Rasagiline treatments. Grade 0: - (0% negative), Grade 1: + (0–33% mild positive), Grade 2: ++ (33–66% moderate positive), Grade 3: +++ (66–100% severe positive).

# Discussion

The pathophysiology of sepsis is based on the immune response, which contains pro-inflammatory and anti-inflammatory processes formed by antigens or toxins of microorganisms entering the body in the host immune system. Many treatments have been tried to block this inflammatory cascade, such as high-dose corticosteroids[29], anti-endotoxin antibodies[30], Tnf-α antagonists[31], IL1 receptor antagonists.[32] The failure to achieve a positive result for reducing mortality with these anti-inflammatory agents aimed at pro-inflammatory activity in sepsis led researchers to the out-of-control inflammation that exists in sepsis.

It is important to trigger the immune response by specific recognition of the microbial component with soluble or pattern recognition molecules such as CD14 and TLR in triggering sepsis.[33]Its effects begin when host defense cells recognize microbial components (e.g. Binding of TLR-2 to gram positive bacterial peptidoglycan). The cytosolic NF-κβ signaling cascade is activated and moves from cytoplasm to nucleus. It binds to transcription sites and induces the gene cluster responsible for the release of pro-inflammatory cytokines. Polymorph nucleus leukocytes are activated. With the help of mediators released from the endothelium, PNL migrates to the inflammation site in a series of steps (rolling, adhesion, diapedesis, and chemotaxis).[34]

When we examined our study, the increase of NF-κβ mRNA expression in sepsis groups and the resulting increase in Tnf-α and IL1β mRNA expressions reveal the severity of inflammation in the lung tissue. As a result of rasagiline administration, it was observed that the increased NF-κβ, Tnf-α and IL1β mRNA expressions in sepsis groups were down-regulated. This shows that rasagiline may be effective by preventing the development of inflammation caused by sepsis. Previous studies show that rasagiline provides cell viability by NF-κβ activation in neurons.[35]However, in our study, it is observed that NF-κβ

mRNA expression, which increased in inflammatory response, was suppressed with rasagiline administration.In this case, it shows that rasagiline can exert different effects in different physiological conditions. In previous studies, rasagiline has been shown to prevent increased IL1β release due to inflammation induced by LPS.[15]Tnfα and IL6 accompany the acute phase response due to sepsis. In sepsis and endotoxic shock, IL6 levels rise to very high levels with Tnf-α.[36, 37] In our study, IL6 mRNA expression was found to be high in correlation with Tnfα in the sepsis group.It is observed that IL6 mRNA expressions were significantly decreased in the groups to which we applied rasagiline. Trudler et al. showed that rasagiline reduced the development of Parkinson's disease by preventing microglial inflammation and in this study, IL6 level decreased after rasagiline administration.[38]

In the late phase of sepsis, inflammatory activation occurs in the continuation of acute inflammation due to bacterial peptidoglycan, dsRNA or endotoxins, resulting in (High Mobility Group Box 1) HMGB1 release from activated macrophages and monocytes.[39, 40]It increases the migration of immune cells to the environment with its chemotactic feature after HMGB1 release. It also increases the inflammasome (NLRP3)response that develops due to sepsis.[39, 41]The main task of NLRP3 is activation of caspase-1.[42]It also serves as a key factor in regulating the production of IL1β in inflammatory processes such as sepsis.While NF-κβ mediated transcriptional increase in the presence of infection is provided by TLR or NOD2 agonists such as lipopolysaccharide (LPS), it has been shown that Tnfα and IL1β or LDL that can bind TLRs under sterile inflammatory conditions can provide this first signal.[42]The second signal necessary for the activation of NLRP3 inflammasome, and subsequent activation of caspase-1 and pro-IL1β cleavage, is provided by stimuli that can specifically activate NLRP3.[42]

Therefore, inflammation that increases in both the early and late phases of sepsis leads to multi-organ failure if not treated.In our study, it is seen that NLRP3 mRNA expressions increased in sepsis groups and HMGB1 mRNA expressions increased in parallel.In rasagiline applied groups, NLRP3 and HMGB1 mRNA expressions were found to be significantly decreased compared to sepsis group. Regarding our subject, Ricardo et al have demonstrated the relationship between monoamine oxidase and NLRP3 inflammation in their study.They showed that the increase in MAO-B expression leads to an increase in NLRP3 level. In particular, they revealed that the NLRP3 level decreased with rasagiline administration. Based on this result, we considered that rasagiline can act by preventing both early and late inflammatory response that develops due to sepsis.

In the last 10–15 years, many studies have been conducted on the relation of oxidative stress, which occurs as a result of the deterioration of the balance between reactive oxygen products and antioxidants, and sepsis.[43]Oxidative stress accompanies inflammation due to sepsis and aggravates tissue damage in these two systems.[44]Many transcription factors, such as increased NF-κβ expression, mainly contribute to the initiation of oxidative stress and inflammation.[45]

At the same time, studies have shown that reactive oxygen radicals accumulated as a result of increased oxidative stress in sepsis lead to NLRP3 activation and increase HMGB1 release.[46]Therefore, the balance between oxidant-antioxidant systems is important for the development of organ dysfunction.

When the lung tissues in the sepsis group are examined, it is seen that the MDA level is quite high. In our study, the accompanying oxidative stress with increasing inflammation reveals the severity of the damage occurred in the lung. In parallel with oxidative stress, SOD and GSH levels, which are the most important components of the antioxidant defense system, decrease in the sepsis group. It was observed that the levels of antioxidant components improved significantly as a result of rasagiline application, while MDA levels were observed to be close to normal.In this regard, rasagiline increased antioxidant activity in lung tissue and prevented lung damage that may develop due to oxidative stress. Rasagiline, which has been chemically shown to have antioxidant activity, has also been shown to reduce oxidative stress in experimental studies. In the experimental MI model, it has been shown that rasagiline administration reduces oxidative stress, and consequently tissue MDA levels decrease.[14]Carrilo et al. showed that rasagiline exerts a neuroprotective effect by increasing SOD and CAT activity in dopaminergic neurons.[47]In this case, rasagiline prevented the organ damage that may develop due to oxidative stress as a result of sepsis both by increasing the antioxidant activity in the lung tissue and by suppressing the expression of NLRP3 and HMGB1 mRNA in the late phase of sepsis.

Finally, in our study, pathological changes occurring in the lung tissue due to sepsis were examined. In systemic inflammatory diseases such as sepsis, the most frequently affected organs are the lungs.[48]More than 10 million case studies by Sands et al show that lungs are the origin of sepsis.[49]Due to sepsis, many pathological events such as hemorrhage, edema, increase in the number of immune cells and pulmonary obstruction accompany the lungs.[50] When we examined our study, it was observed that edema, hemorrhage and inflammation focuses were moderate in sepsis groups, while edema, inflammation and hemorrhagic areas almost completely disappeared in the groups to which rasagiline was applied.

## Conclusion

Rasagilin exerts both antioxidant and anti-inflammatory effects on CLP induced acute lung injury in rats. Therefore, its lead to an increase impact on the MAO-B inhibitors both for the treatment of neurodegenerative diseases such as parkinson and other inflammatory diseases.

## Declarations

# Ethics approval and consent to participate:

This study was approved by and performed in accordance with the institutional animal care and use ethics committee of Ataturk University with the protocol number 2019-6/98

# Consent for publication:

Yes

# Availability of data and materials:

Yes

# Competing interests:

There is no conflict of interest

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# Authors' contributions:

Design and Final Revisions: Harun Un, Zekai Halici, Methods and Animal Procedure: Harun Un, Rustem Anil Ugan, Duygu Kose, Muhammed Yayla, Molecular Analyses: Rustem Anil Ugan, Muhammed Yayla, Histological Analyses: Tugba Bal Tastan, Biochemical Analyses: Yasin Bayir.

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# Figures

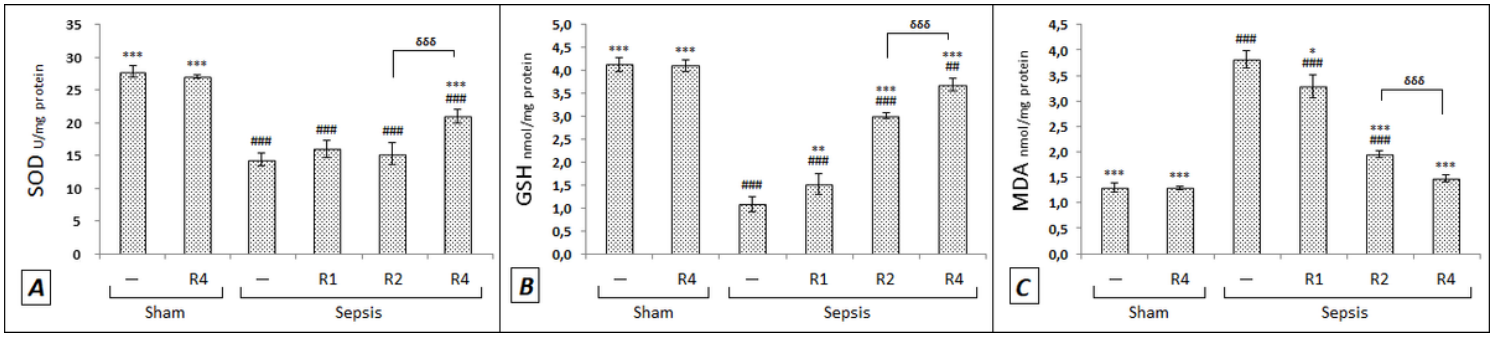
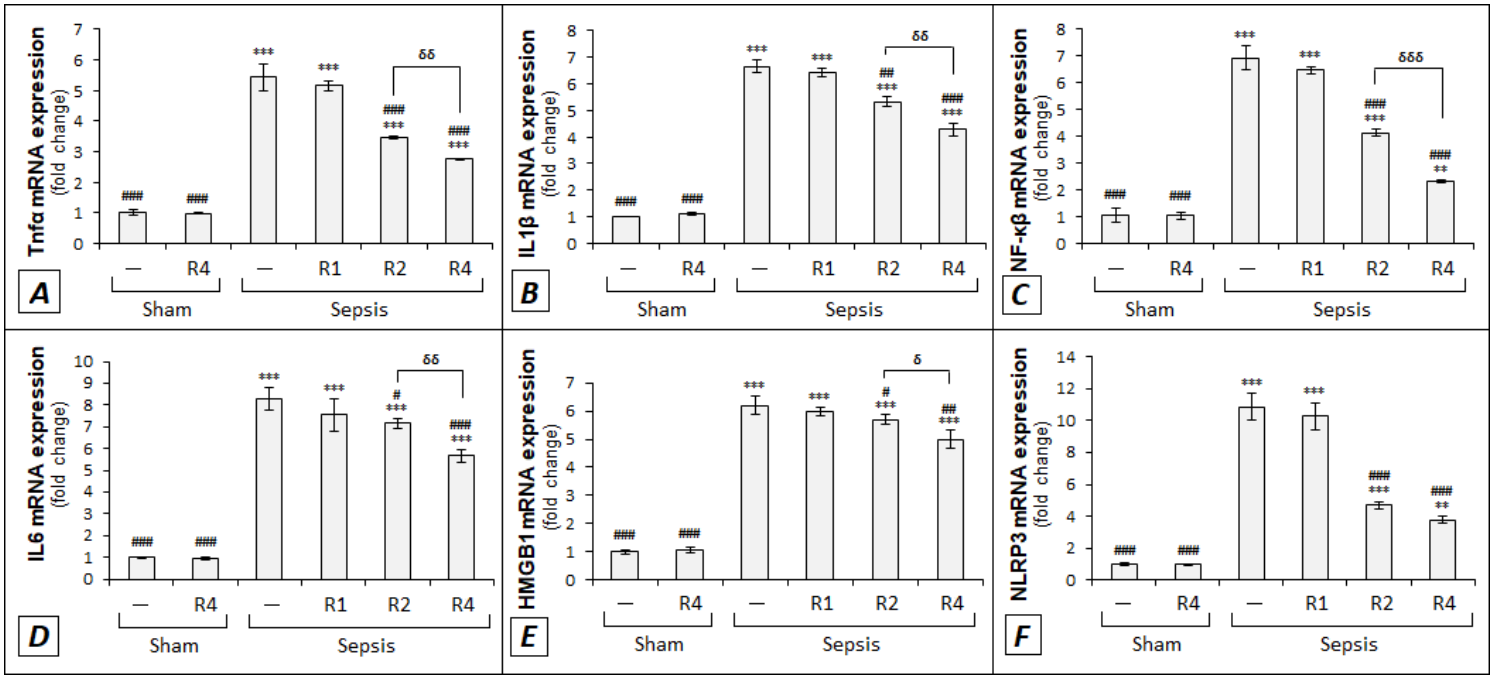


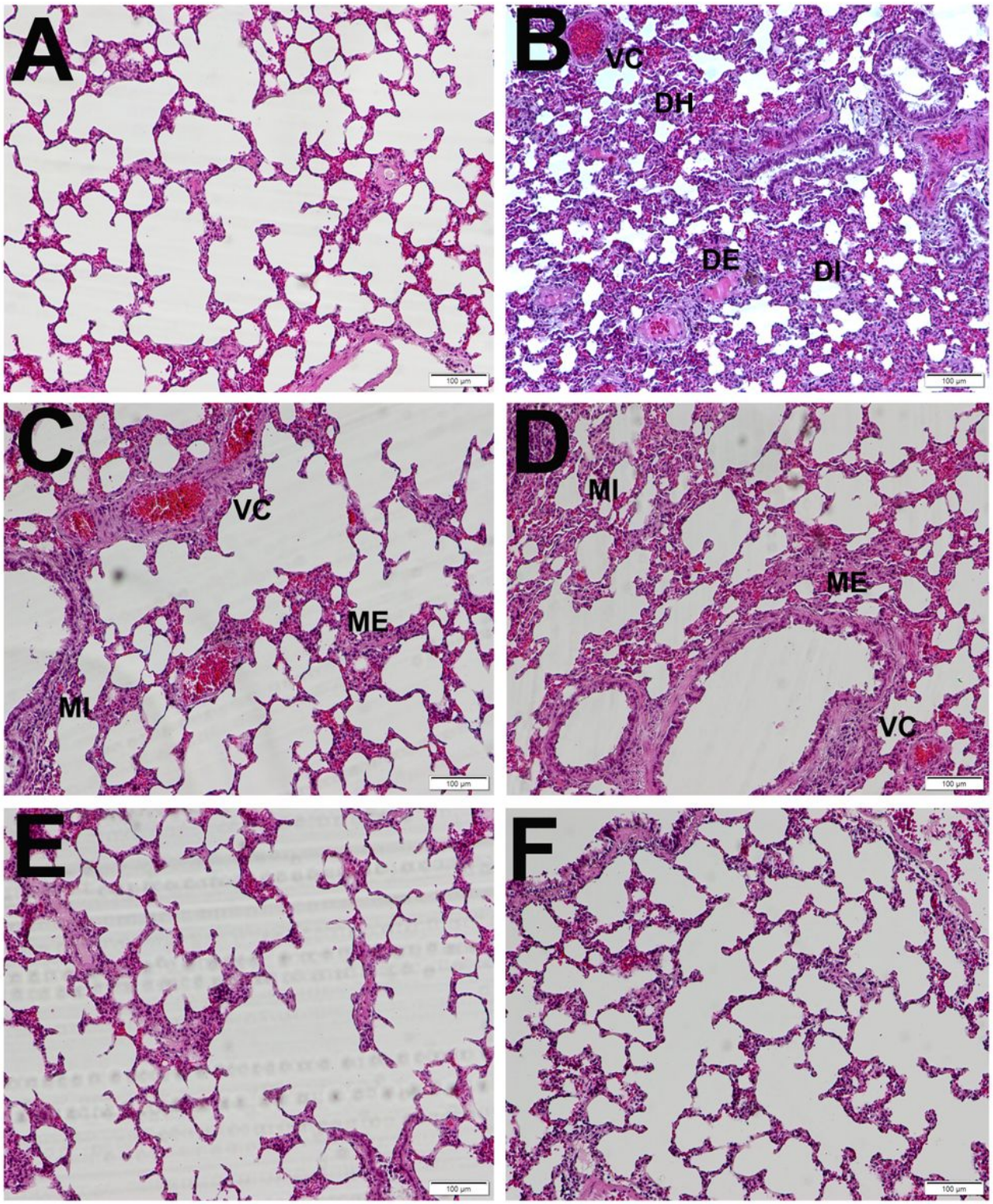

**Figure 1**

Biochemical results of Rasagiline treatments in the lung tissues. R1: 1 mg/kg Rasagiline, R2: 2 mg/kg Rasagiline, R4: 4 mg/kg Rasagiline treatments. GSH: Total glutathione levels, MDA: Malondialdehyde levels, SOD: Superoxide dismutase activities. Each bar expressed as mean value ± SD. Significant differences were detected between all groups, compared to Shamg roup (##p<0.01, ###p<0.001),compared to Sepsis group (*p<0.05, **p<0.01, ***p<0.001) and compared to Sepsis+R4 group(δδδ P<0.001)by one-way ANOVA followed by Tukey test.




## Figure 2

Relative mRNA expression levels of Tnf-α, IL1β, NF-κβ, IL6, HMGB1 and NLRP3 in the lung tissues. R1: 1 mg/kg Rasagiline, R2: 2 mg/kg Rasagiline, R4: 4 mg/kg Rasagiline treatments. The expression of mRNAs was detected using quantitative Real-Time PCR analysis. β-actin was used as the reference gene. Results are expressed as relative fold compared with Sham animals. Each bar expressed as mean value ± SD. Significant differences were detected between all groups, compared to Sham group (*p<0.05, **p<0.01, ***p<0.001), compared to Sepsis group (#p<0.05, ##p<0.01, ###p<0.001) and compared to Sepsis+R4 group(δ P<0.05, δδ P<0.01, δδδ P<0.001)by one-way ANOVA followed by Tukey test.




## Figure 3

Histopathological results of lung tissues with Haematoxylin and Eosin staining. A:Sham, B:Sepsis, C: Sepsis+R1 (1 mg/kg Rasagiline), D: Sepsis+R2 (2 mg/kg Rasagiline), E: Sepsis+R4 (4 mg/kg Rasagiline), F:Sham+R4 (4 mg/kg Rasagiline). ME: Mild edema, DE: Dense edema, MI: Mild inflammation, DI: Dense inflammation, MH: Mild hemorrhage, DH: Dense hemorrhage, VC: Vascular congestion.