BEZMİÂLEM
VAKIF ÜNİVERSİTESİ

Investigation of the relationship of rosuvastatin and atorvastatin with the NRLP3 inflammasome complex in LPS-induced neuroinflammation

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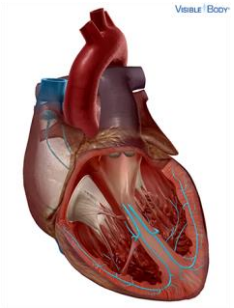
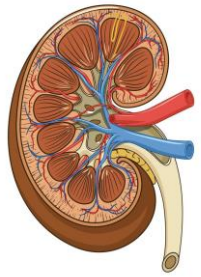
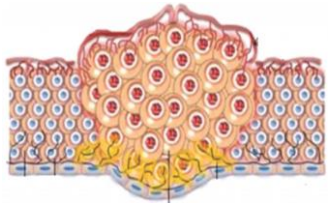
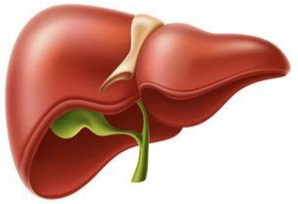
Where Are Statins Used?

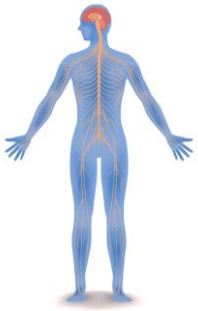
- Statins are a class of cholesterol-lowering drugs that are very frequently used in the treatment of dyslipidemia.







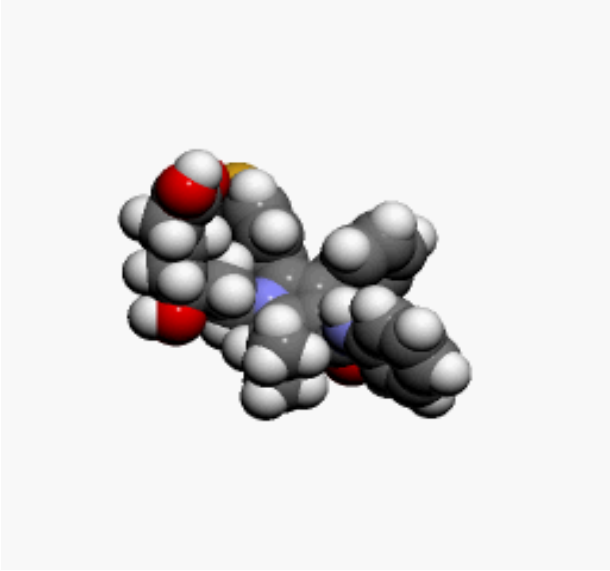




Cardiovascular system (CVS)	Renal diseases	Types of Cancer	Liver diseases	Nervous system

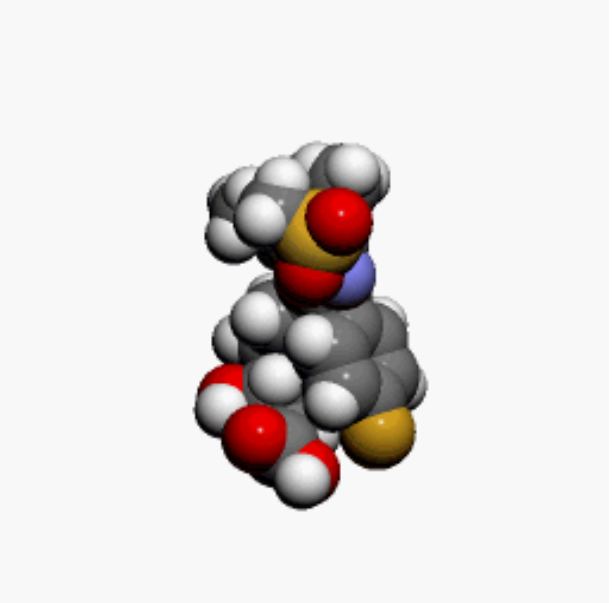
Statins And Nervous System

- Synaptic transmission
- Cognition and memory pathways
- Consolidation phase of the memory process
- Regulation of long-term memory
- Regulation of genes involved in memory and learning
- Neuronal differentiation
- Adult neuritogenesis



Atorvastatin

- Atorvastatin belongs to a group of medicines called statins. It is used to lower cholesterol and prevent heart disease, including heart attacks and strokes.
- ATARVOSTATIN ➜ ANTI-INFLAMMATORY (inhibition of NPRL3 inflammation)

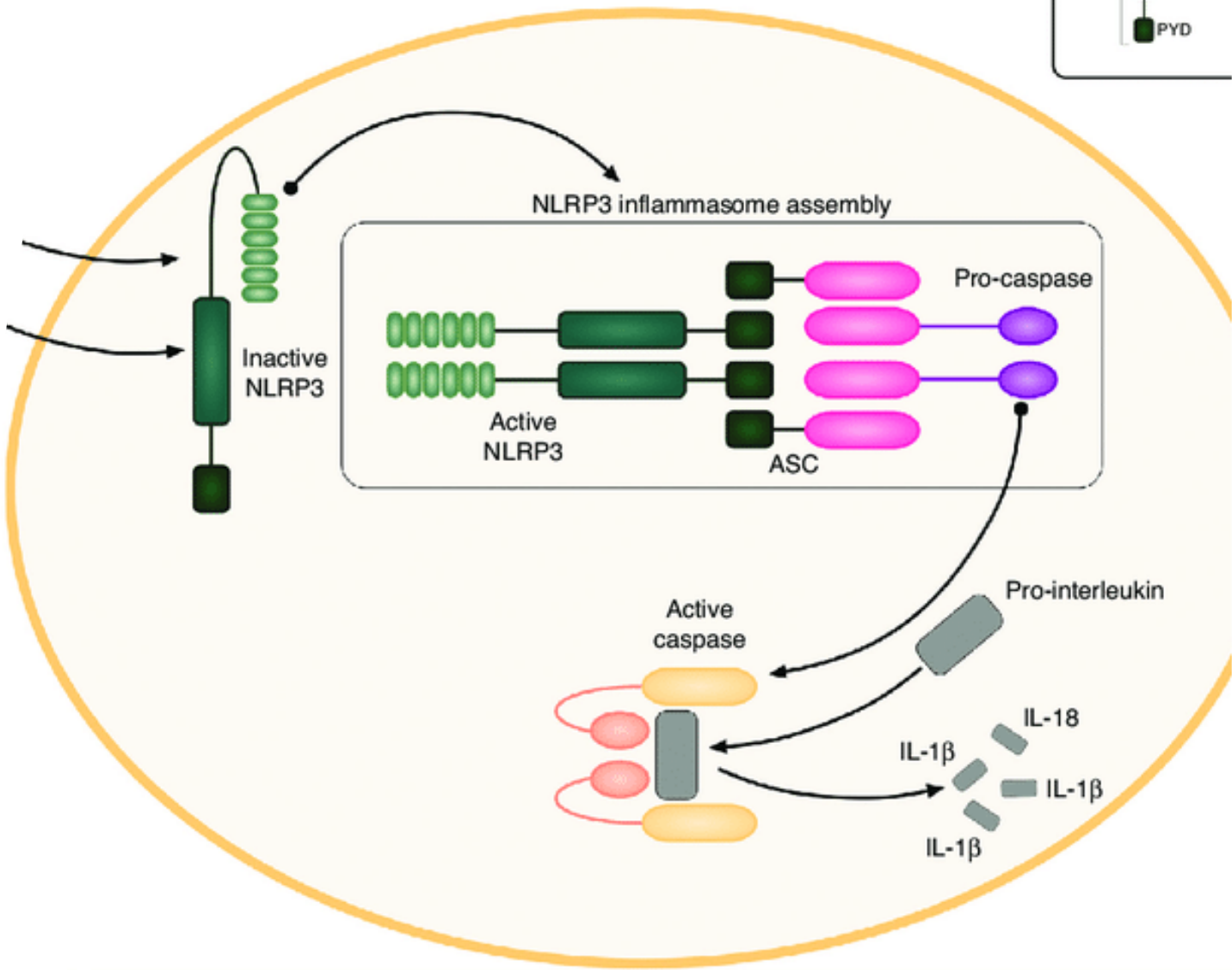


Rosuvastatin

- ROSUVASTATIN ➜ Periprocedural myocardial infarction (PMI)

NLRP3 inflammasome and Neuroinflammation

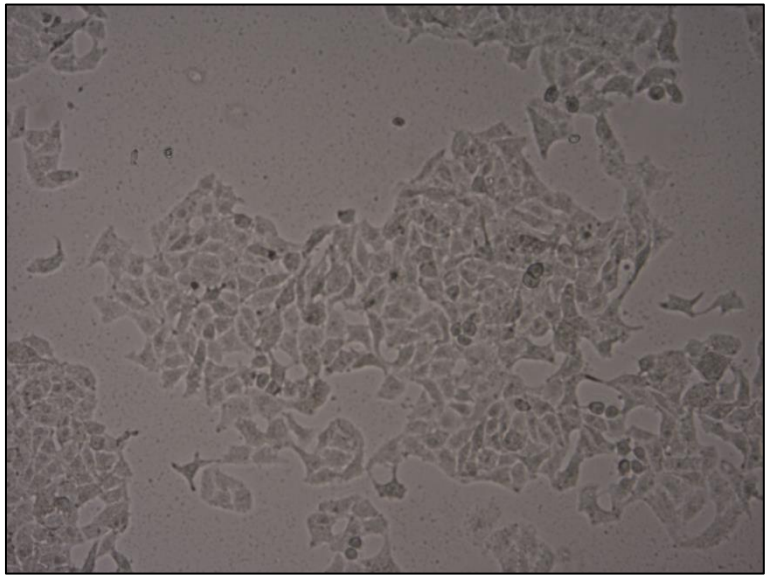
- NLRP3; It is a cytosolic receptor protein that recognizes danger signals reaching immune system cells such as macrophages and microglia and is involved in the initiation of IL-1β and IL-18 mediated inflammatory responses. Inflammation is controlled by the NLRP3 inflammasome, which consists of the NLRP3 protein, procaspase-1, and ASC. NLRP3 is distinguished from other members by having a wide range of distress signal recognition.

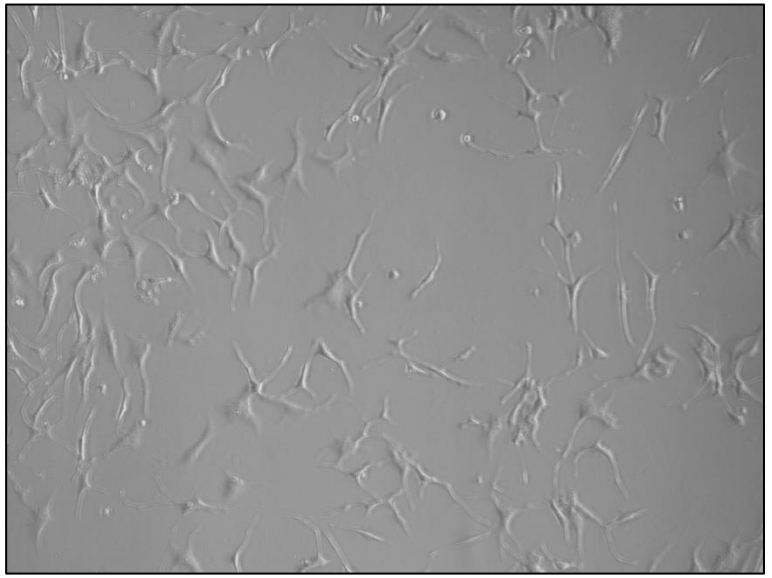


Aim

- Our aim is to understand the effects of rosuvastatin and atorvastatin on the neuroinflammation process to explain whether this process is related to the NRLP3 inflammasome complex.

Material & Method
Cell culture and Differantiation





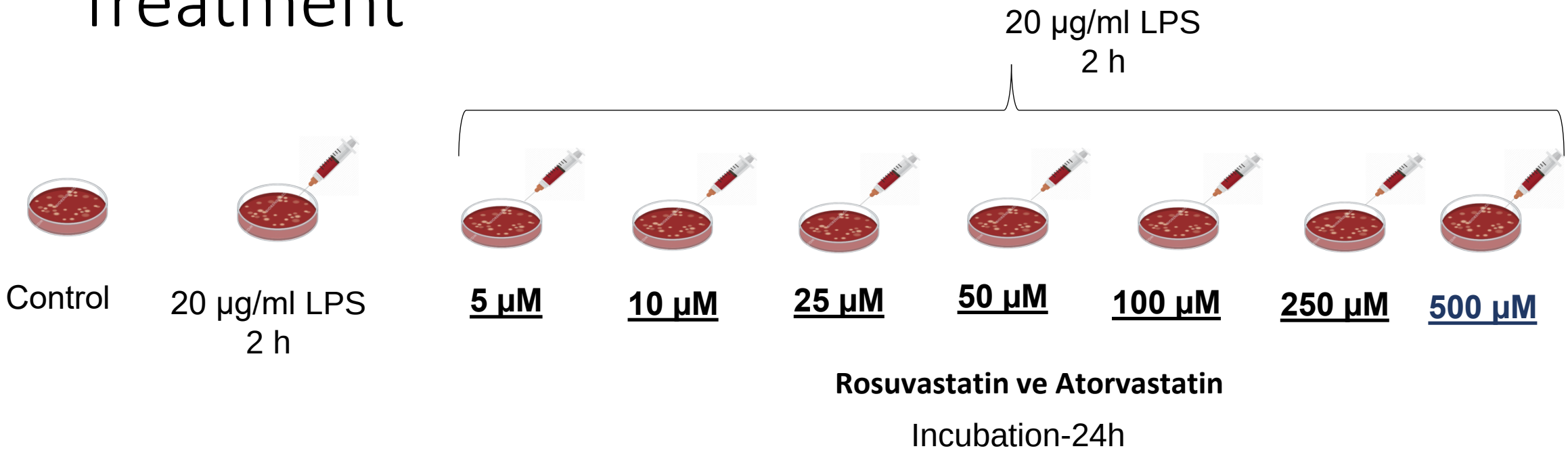
SHSY-5YCell line
(ATCC® ATCC HTB-11)

- The human neuroblastoma SH-SY5Y cell line
- DMEM-F12
- %10 fetal bovine serum (FBS)
- %1 penicillin-streptomycin
- %1 amphotericin B
- 37 °C
- %5 CO_2

- Neuron like cells
- serum-free neurobasal medium
- %1 penicillin-streptomycin
- Retinoic acid (RA)
- In dark
- 37 °C
- %5 CO_2

Differentiation of the SH-SY5Y Human Neuroblastoma Cell Line, Mackenzie M. et al.

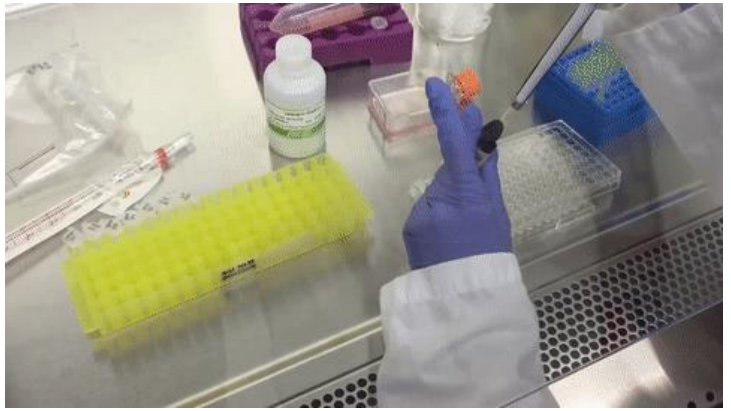
Material & Method
Treatment

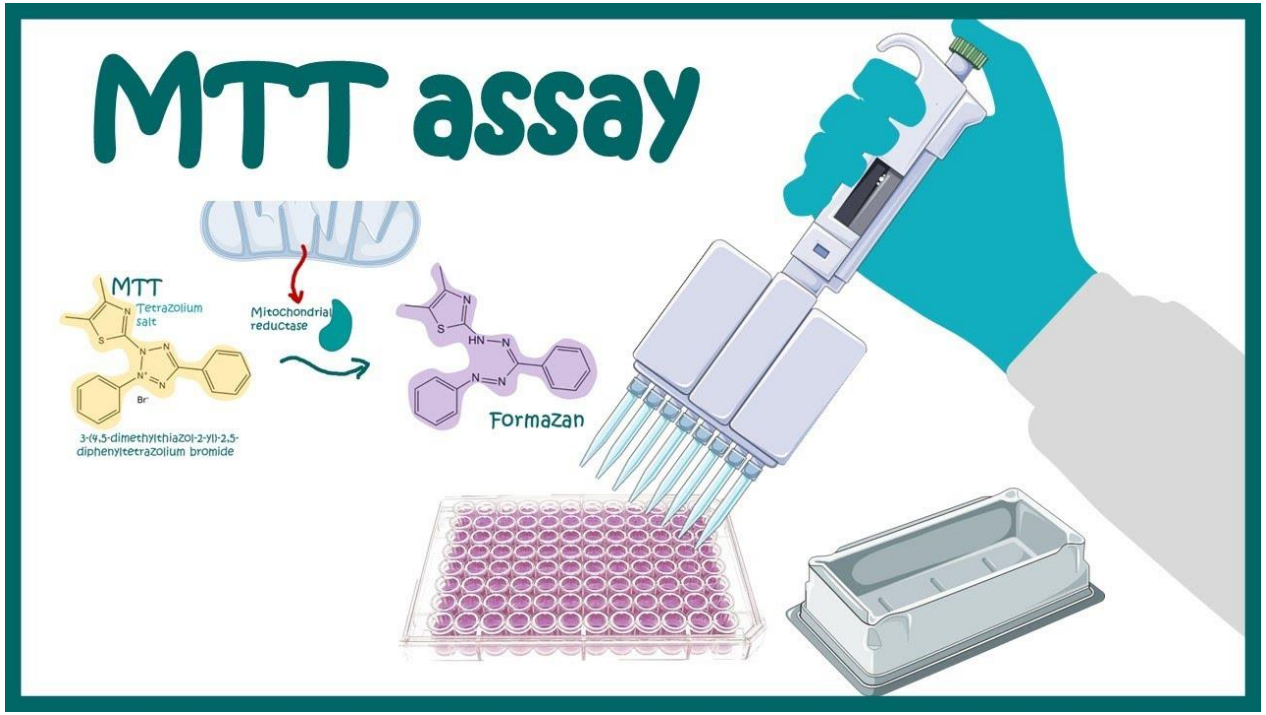


20 µg/ml LPS was applied to the cells that differentiated into the neuron to induce inflammation and incubated for 2 hours.

Then, by using the doses used in the literature, increasing concentrations (5, 10, 25, 50, 100, 250 and 500 µM) Rosuvastatin and Atorvastatin were applied separately and kept for 24 hours, and viability analyzes were performed.

Material & Method
Cell Viability-MTT Assay



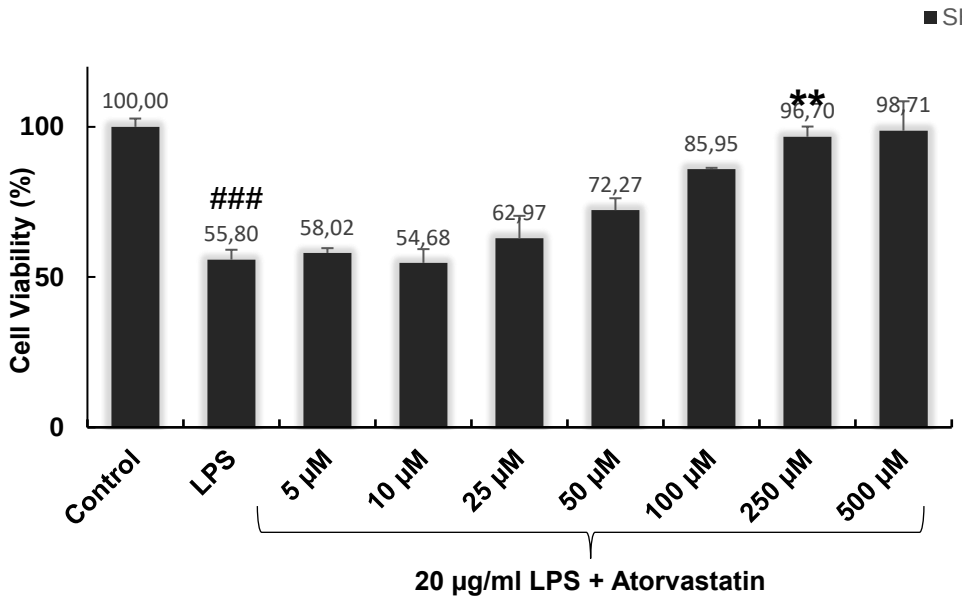


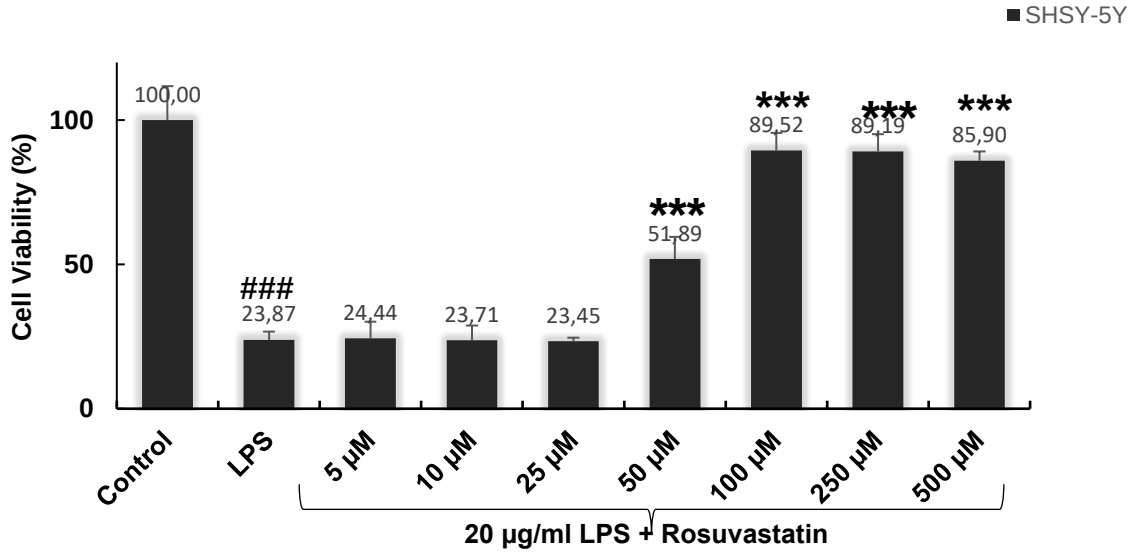
- The viability of cells was tested using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
- Cells were incubated at 37 °C with 0.1 mg/mL MTT for two hours. The supernatants were decanted without being dispensed to the cells.
- Then, 100 µL DMSO was added to the cells, which were then kept in the dark.
- After 30 minutes, optical densities were measured using a microplate reader) at 570 nm. The cell viability assay for each group was performed in triplicate.

Results
Cell Viability

As expected, treatment of LPS alone significantly decreased the cell viability in comparison with the control ($p<0.001$)

Atorvastatin and Rosuvastatin decrased LPS-induced cell death in the differentiated SH-SY5Y cell line.



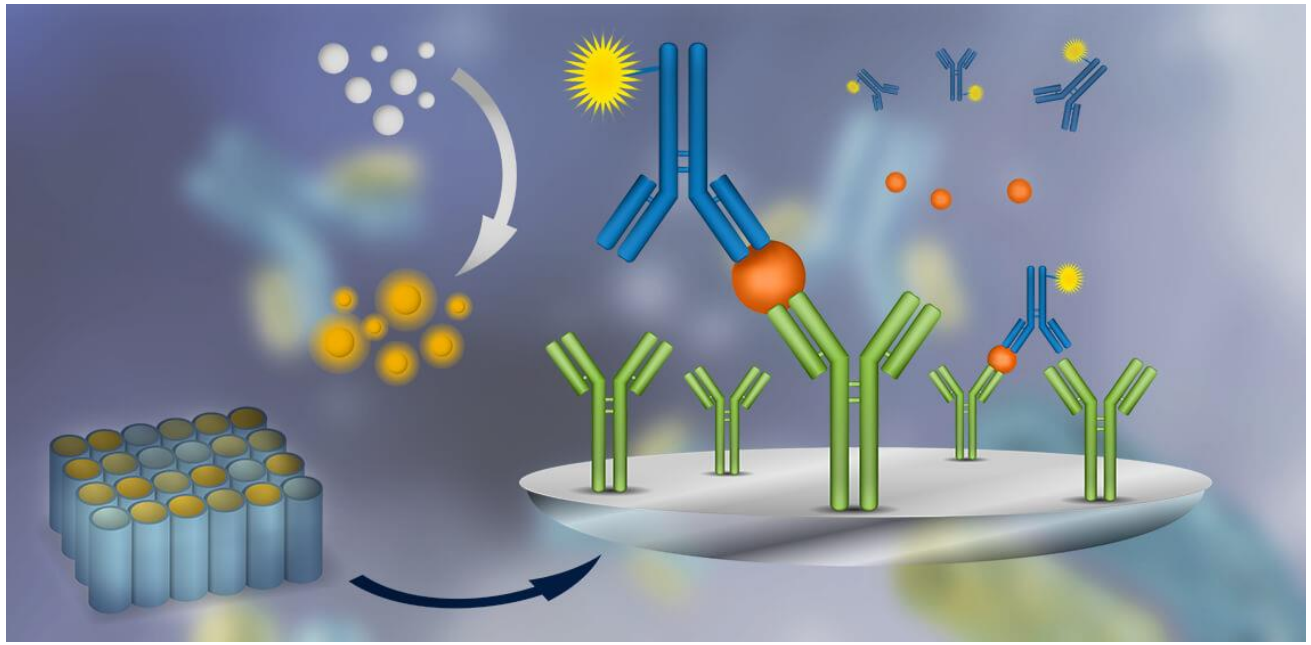


Significant differences compared to control
#= $p<0.05$
##= $p<0.005$
###= $p<0.001$

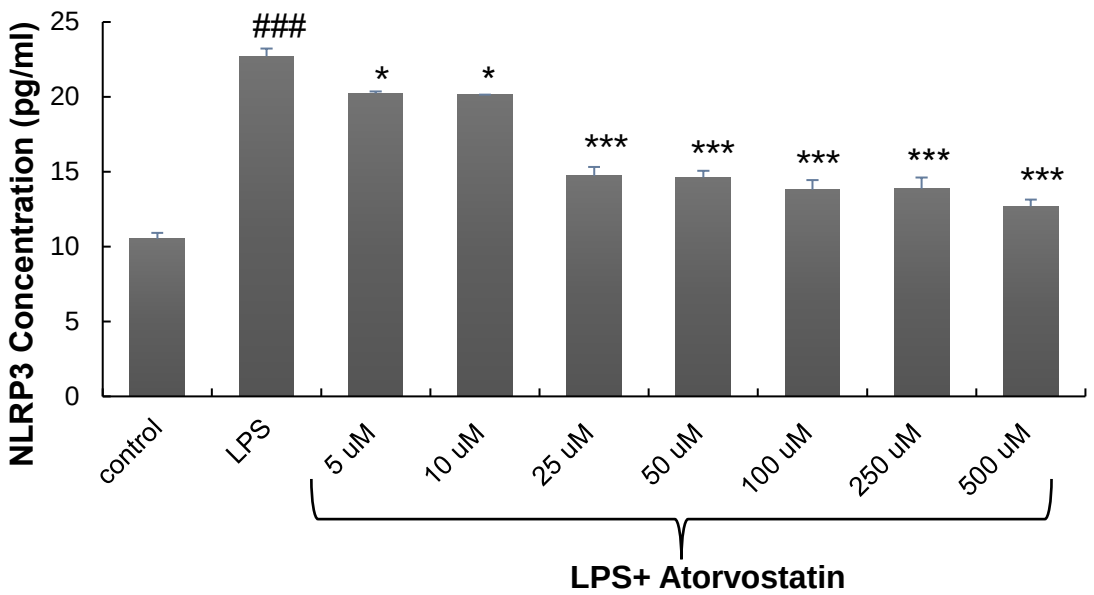
Significant differences compared to LPS
*= $p<0.05$
** = $p<0.005$
*** = $p<0.001$

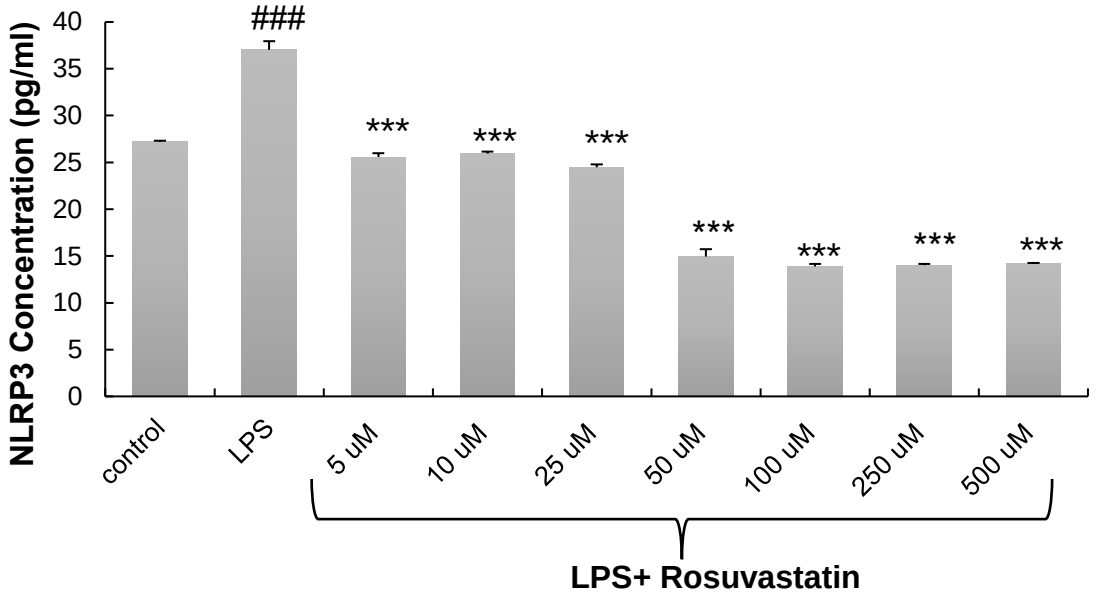
Material & Method
ELISA

Protein amounts of NLRP3 and PYCARD, which are associated with inflammasome formation, were analyzed by ELISA.



Results
ELISA-NLRP3





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Significant differences compared to LPS
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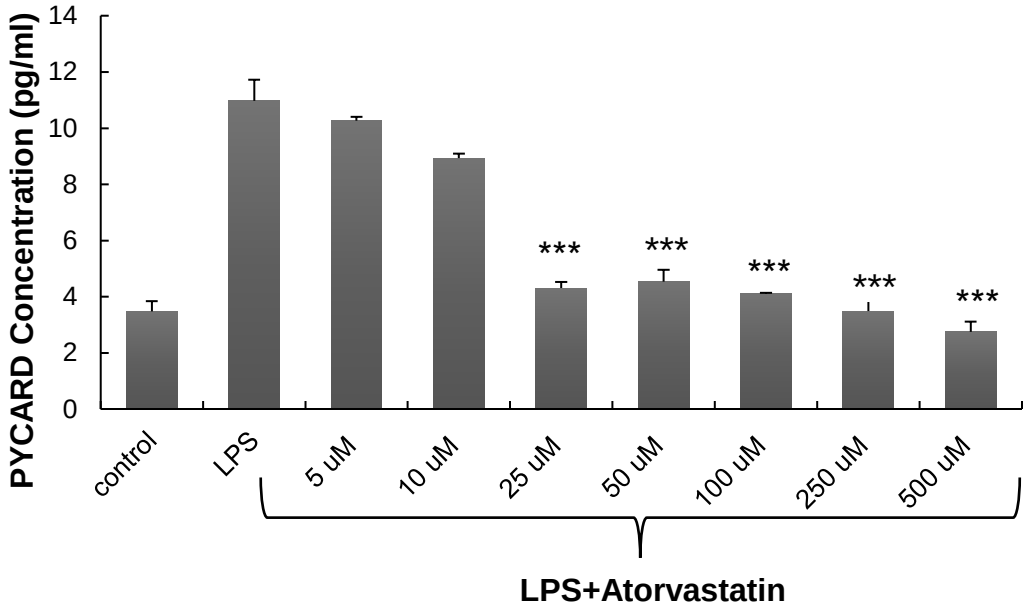
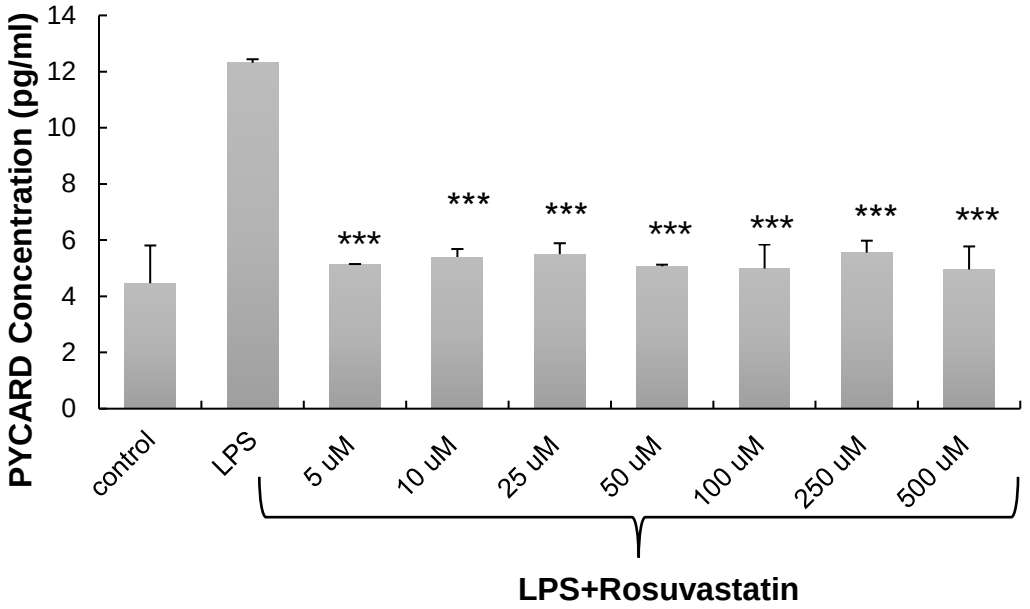
Results
ELISA-PYCARD





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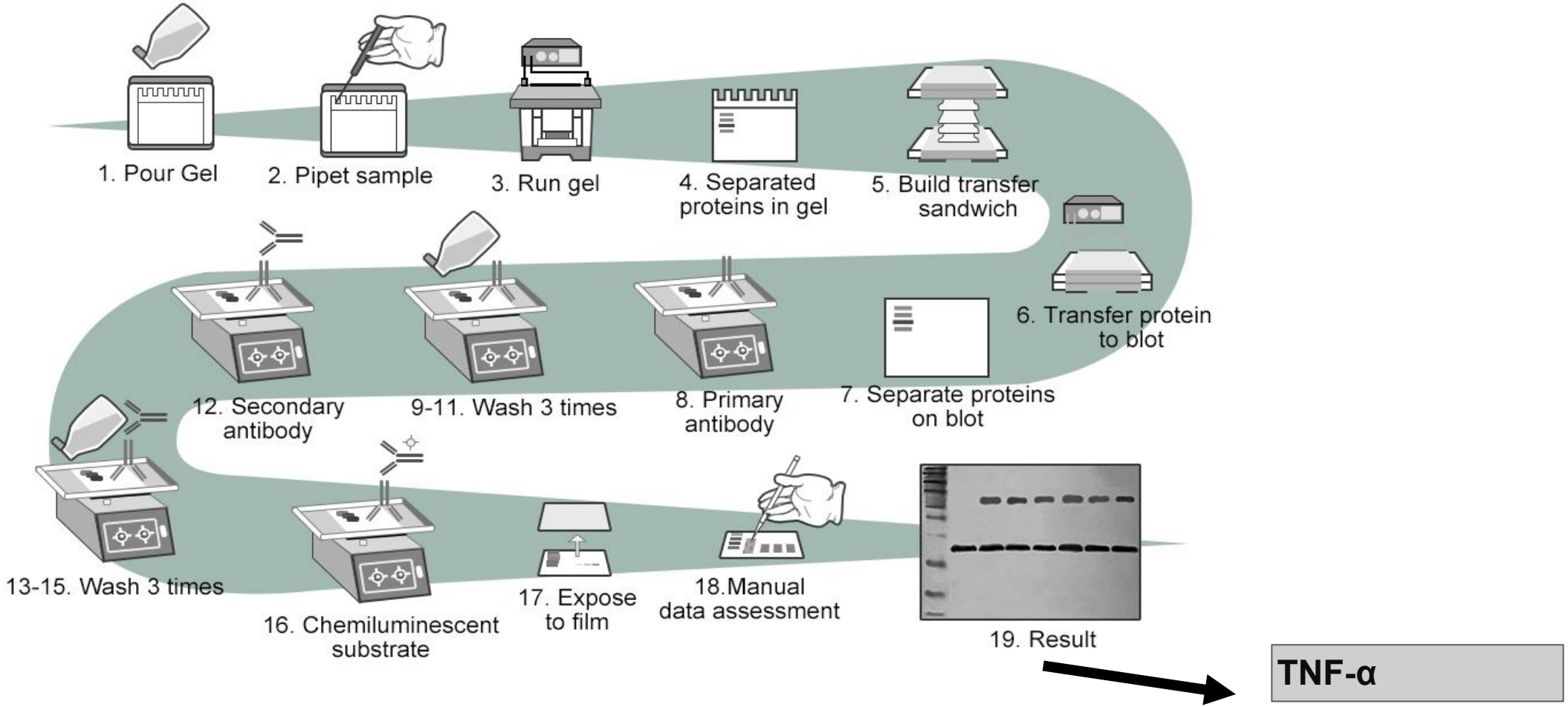
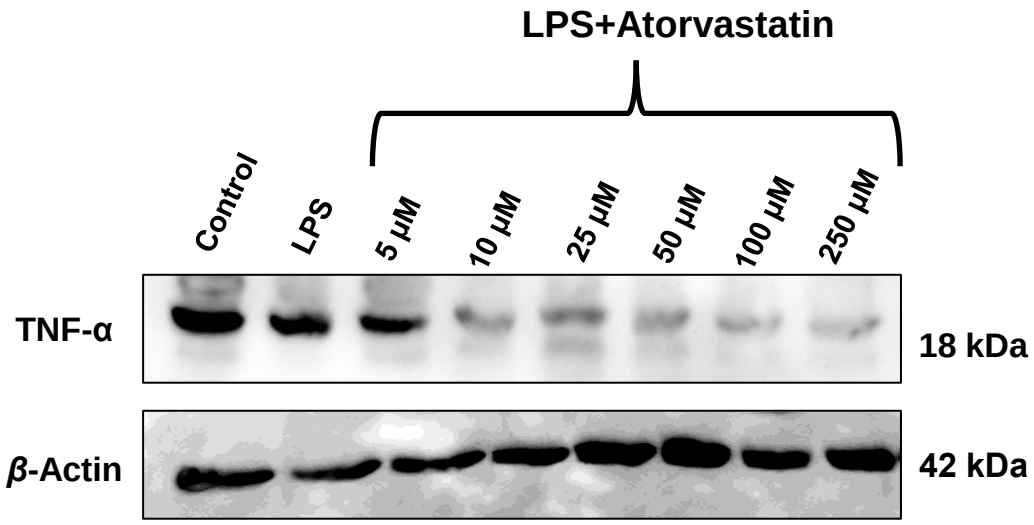
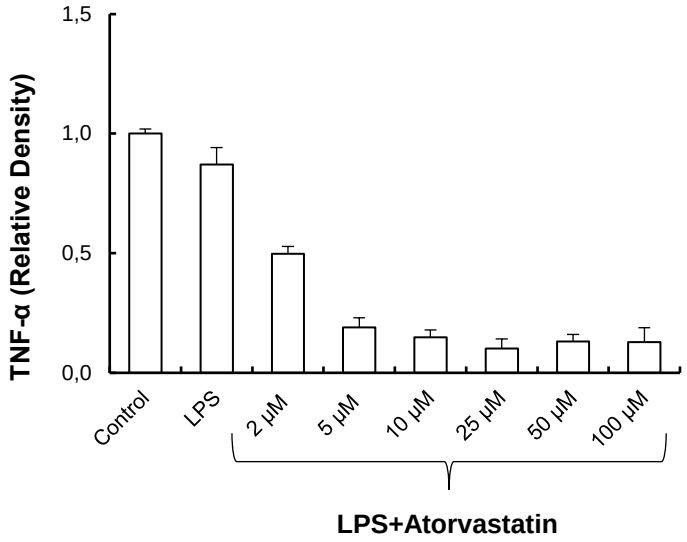
Material & Method
SDS-PAGE and Western Blot



Results
SDS-PAGE and Western Blot





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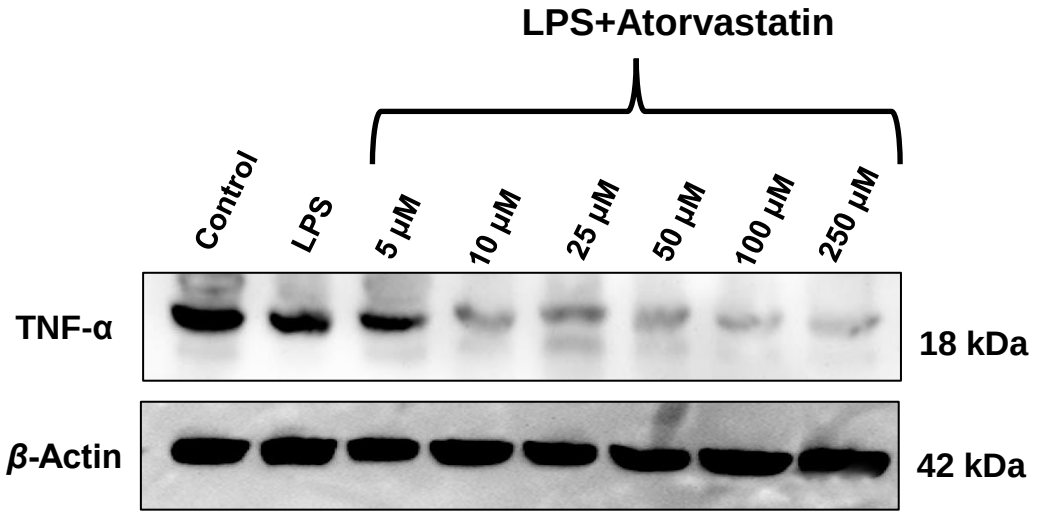

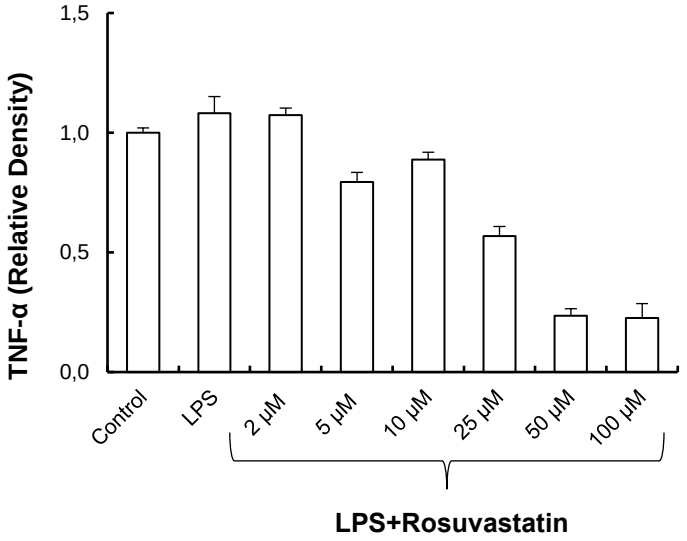

Discussion

- There is a significant recovery was observed both atorvastatin and rosuvastatin treatment separately on LPS-induced neuroinflammation.
- Although, this recovery of cells was observed on 50 µM with atorvastatin treatment, in rosuvastatin treatment group, it needs higher concentration (100 µM). Besides, it's observed that, both atorvastatin and rosuvastatin treatment reduced increased expressions of NLRP3 and PYCARD by LPS to control levels.
- Besides, treatment of atorvastatin and rosuvasatin reduced expresion of some inlflammational proteins (TNF-α)on LPS-induced neuroinflammation.
- Based on these results, it will be studied molecular mechanism of atorvastatin and rosuvastatin on neuroinflammation and relation of NLRP3 inflammasome complex.

Limitations

- Limited financial support ☺
- Limited time of study

Summary

- Atorvastatin and rosuvastatin showed improvement in cell viability in neuroinflammation of LPS-induced neuron like cells.
- Atorvastatin and rosuvastatin administration of NLRP3 and PYCARD (ASC) protein expressions, which are involved in the formation of NLRP3 inflammasome complex, are decreased.

References

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Thank you

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