

A Simple Methods for Non Phenolic Extraction of Lipopolysaccharide From *Salmonella enteritidis* and Effect of Extracted Lipopolysaccharide on Mesenchymal Stem Cells

Nafiseh Asgarzade*¹; Hossein Rastegar²; Hamidreza Ahamdi Ashtiani³; Mehdi Hedayati⁴

1-Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

2-Food and Drug Control Laboratory and Research Center, Tehran, Iran

3-Department of Clinical Biochemistry, School of Medical Science, Tarbiat Modarres University, Tehran, Iran

4-Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

nafisehasgarzade@yahoo.com

Background & Objectives: Lipopolysaccharide (LPS) is the most outer part of gram negative bacteria cell wall which is connect bacteria to its environments. LPS is the main determining factor of gram-negative bacterial virulence. It releases via bacterial lysis and called endotoxine. LPS is a strong stimulator of immune responses. Its connection to endothelial cells and macrophages activates immune system regulatory cells. Following this process, synthesis of various intermediate compounds such as IL-1beta and TNF-alpha increase which have important role in host cells defense against gram-negative bacterial infections. This compound can cause severe reactions in the immune system. Pure LPS alone is able to induce strong inflammatory reactions. Nitric Oxide is an important molecular marker in many tissues which produces by Nitric oxide synthetase. iNOS is inducible isomer of Nitric Oxide synthetase. In order to killing bacterial cell, immune dependent cells such as macrophages produce a lot of Nitric Oxide, then NO determination is a reason of LPS effect assessment on immune system, NO was determined.

Methods: *Salmonella enteritidis* suspension cultured in Caso broth media and LPS extracted by Methanol-Chloform Methods. It added to Mesenchymal cell culture in DMEM contains FBS, antibiotics and CO₂. LPS with 100ng affect ed the cells for 4hrs and then iNOS level was determined by iNOS assay kit.

Results: LPS extracts with methods which is introduced here showed high purity in comparison to standard LPS by running on SDS-PAGE. The initial amount of iNOS before stimulation was 10.94U/ml. In the group with LPS stimulation, iNOS increased to 16.09U/ml.

Conclusion: According to the results it is deduced that this methods is very convenient, cost-effective, efficient, and safe in comparison with other methods. Based on the results, LPS can stimulate human immune system and inflammatory *cofactors* and finally increase NO production.

Keywords: Non Phenolic Extraction; Lipopolysaccharide; Mesenchymal Stem Cells