Possible Protective Effects of Montelukast against Endotoxic Effect of Lipopolysaccharide in Bone Marrow and Spleen in Experimental Male Mice. Comparison Study with Dexamethasone

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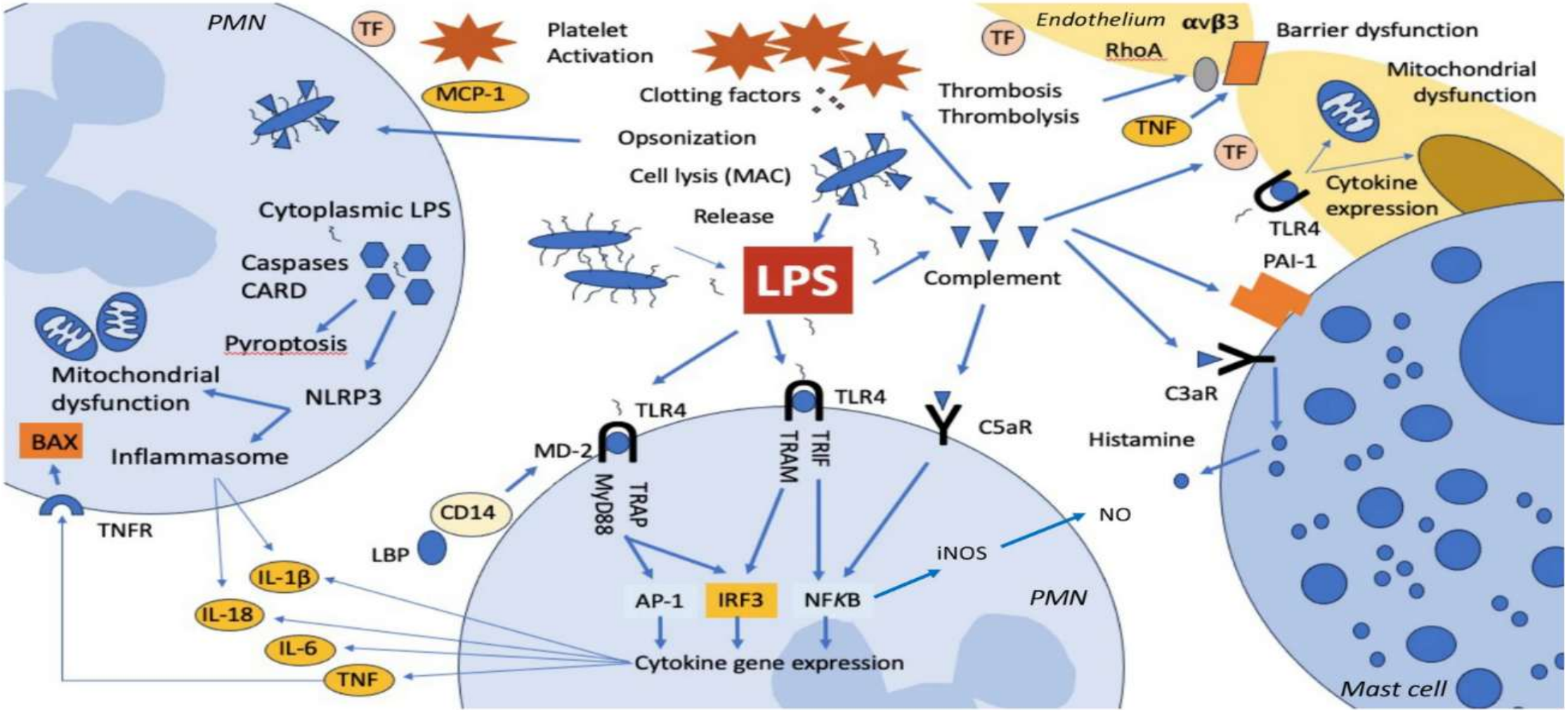
Introduction

- Endotoxic septic shock (ESS) is a serious clinical syndrome caused mostly by gram-negative bacterial infections. ESS refers to infected patients with high bloodstream endotoxin levels and activity with a high rate of cardiovascular, pulmonary, hepatic, and renal failure; Besides, ESS can be reserved for patients who can benefit from anti-endotoxin therapy.
- The endotoxin in the bacterial cell wall can evoke neutrophils, and monocytes and secrete inflammatory mediators like cytokines, nitric oxide, histamine, and bradykinin, causing local and systemic inflammation, severe vasodilation, endothelial dysfunction, increased capillary permeability, and disseminated intravascular coagulation.

Introduction

- Moreover, the prooxidant-antioxidant imbalance can cause endocrine, cardiovascular, neurological, respiratory, renal, and hepatic disorders and may lead to organ(s) failure in septic patients.
- Mice were utilized as experimental animals for research by induction of endotoxemia/septic-like condition that closely simulates septic shock in humans via administering lipopolysaccharide (LPS) isolated from a particular bacterial strain at an appropriate dose.
- Toll-like receptors (TLRs) are the primary receptors targeted by LPS, and can be a vital target for prophylactic or therapeutic agents.

Mechanism of LPS-induced endotoxic shock



Problems

- Despite significant progress in the knowledge of sepsis's pathophysiology, immuno-inflammatory pathways, and causes over the past few decades, there is still a rapid deterioration in septic patients, and increased mortality rates remain dilemmatic, together with a lack of safe and successful treatments for instance, Anakinra (an IL-1 receptor antagonist) and eculizumab (a monoclonal antibody to C5) may be unsafe for sepsis patients if the infection is still active.

- Endotoxin-neutralizing proteins, Lipid A analogs, and alkaline phosphatase have failed in clinical studies. It is worthwhile to investigate the possibility of using either novel or old xenobiotics that can modulate intracellular molecular pathways and exaggerated inflammatory cascade reactions without worsening patients' conditions, and some patients may respond to therapies out of sepsis bundles routine; This topic has attracted the attention of many researchers.

Aim of the study

- This study was designed to explore the possible protective effect of montelukast in comparison with dexamethasone on bone marrow and spleen of male albino mice from the LPS-induced endotoxicity by measuring the following points:

- 1. Inflammatory markers in serum such as TNF-α and IL-6 utilizing Enzyme-linked immunosorbent assay (ELISA) techniques.

- 2. Bone resorption markers such as receptor activator nuclear factor kappa B (RANK), and osteoprotegerin (OPG) in serum.

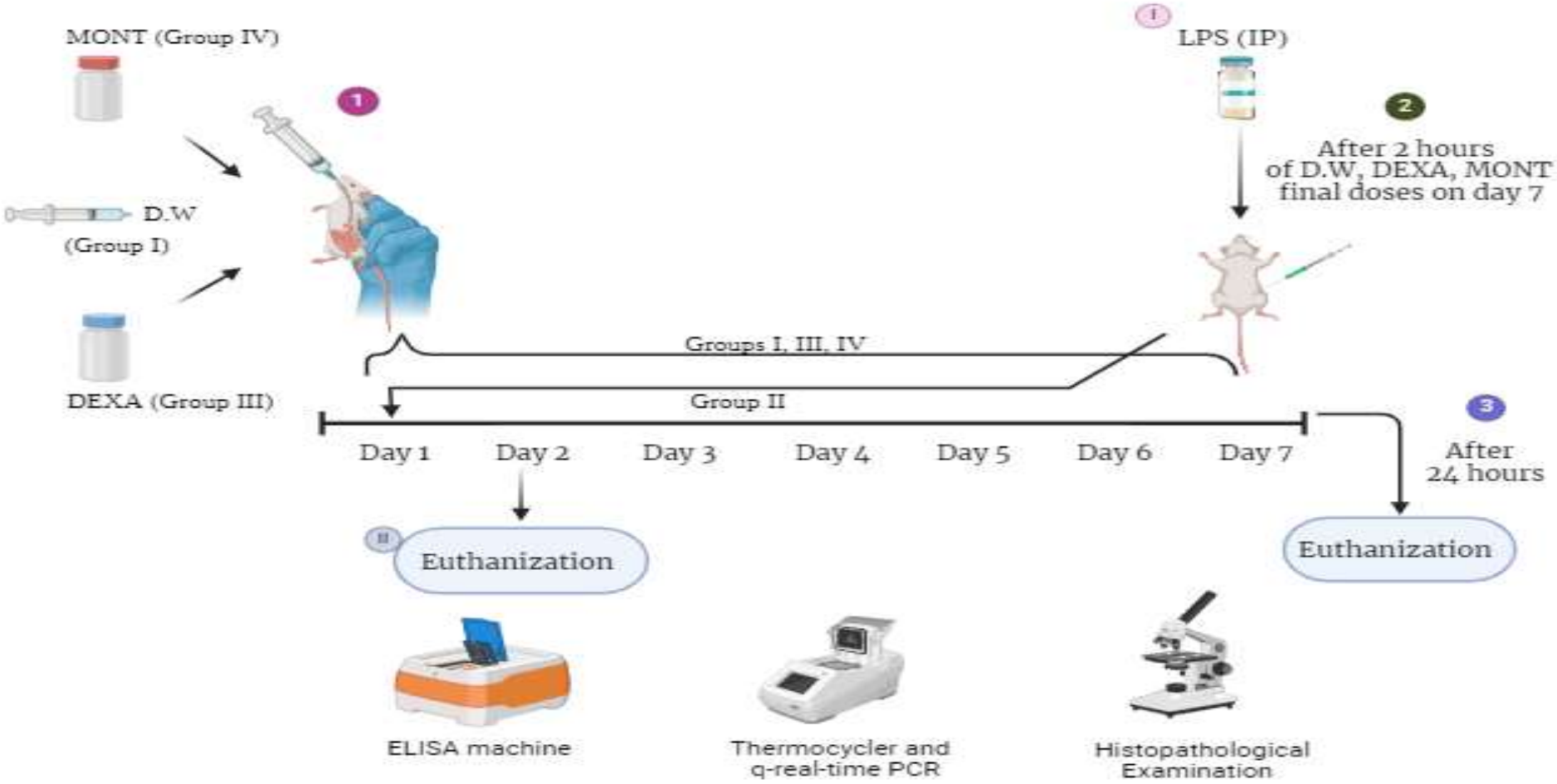
Aim of the study

- 3. Oxidative stress markers in bone marrow such as myeloperoxidase (MPO) activity and malondialdehyde (MDA) utilizing ELISA techniques.

- 4. Fold change in each cathepsin-G (CTG) and neutrophil elastase (NE) in relation to a reference gene β-Actin in the spleen utilizing quantitative real-time-polymerase chain reaction (qrt-PCR) techniques.

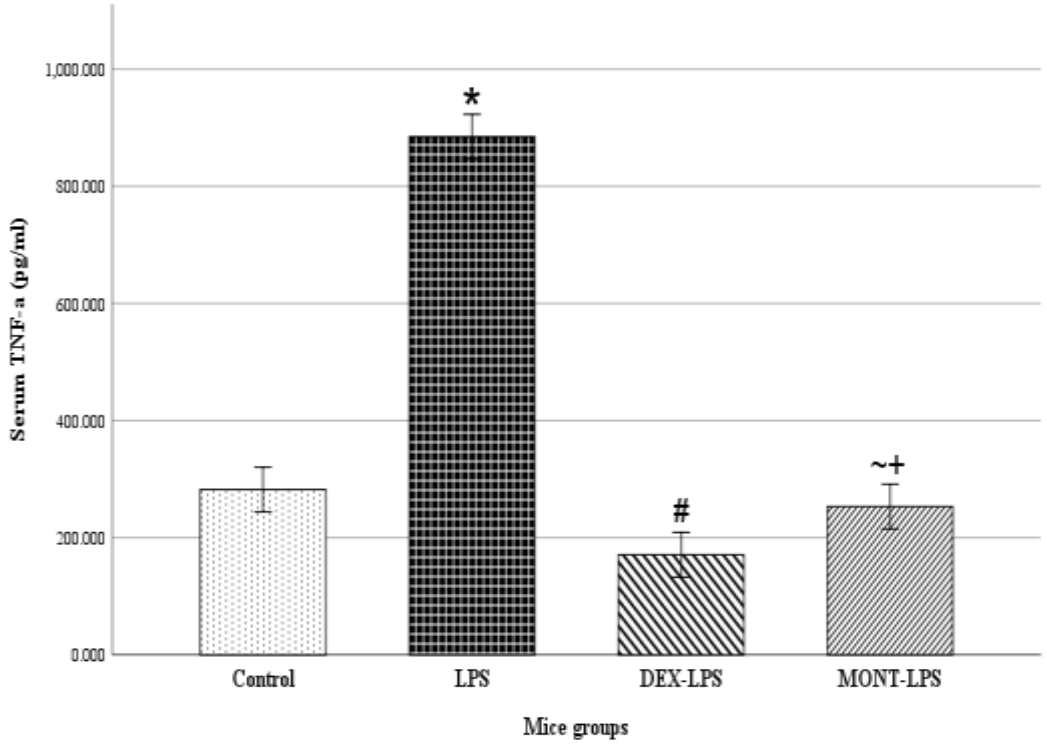
- 5. Histopathological changes in bone marrow.

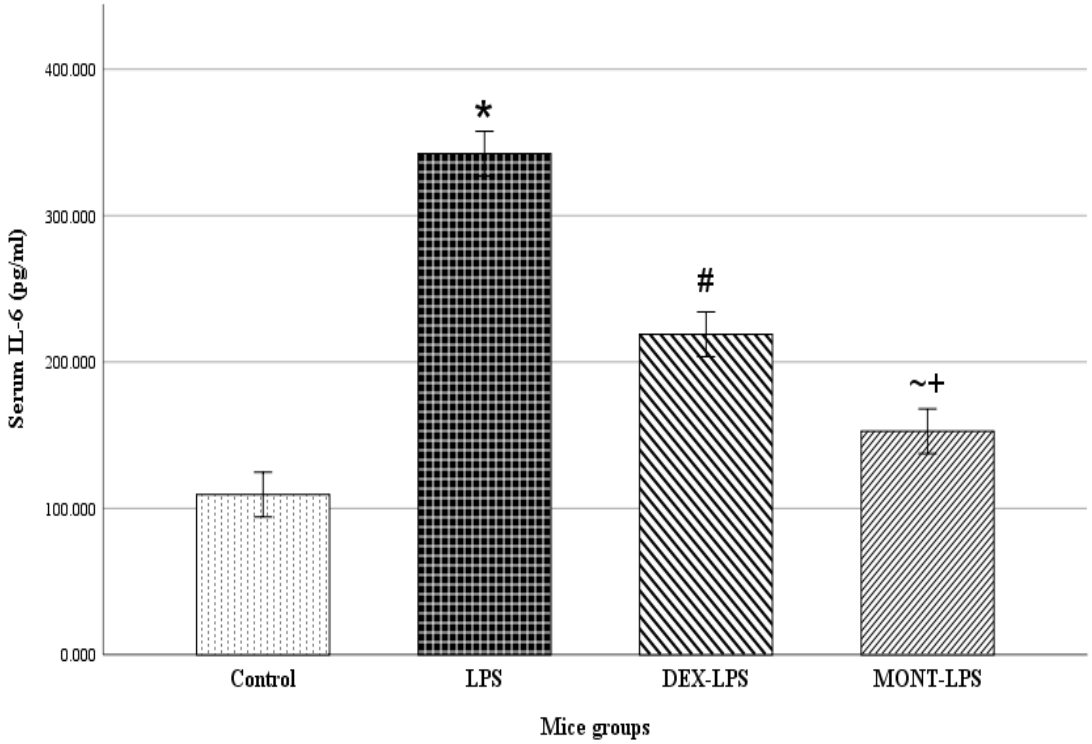
Experimental protocol



Results

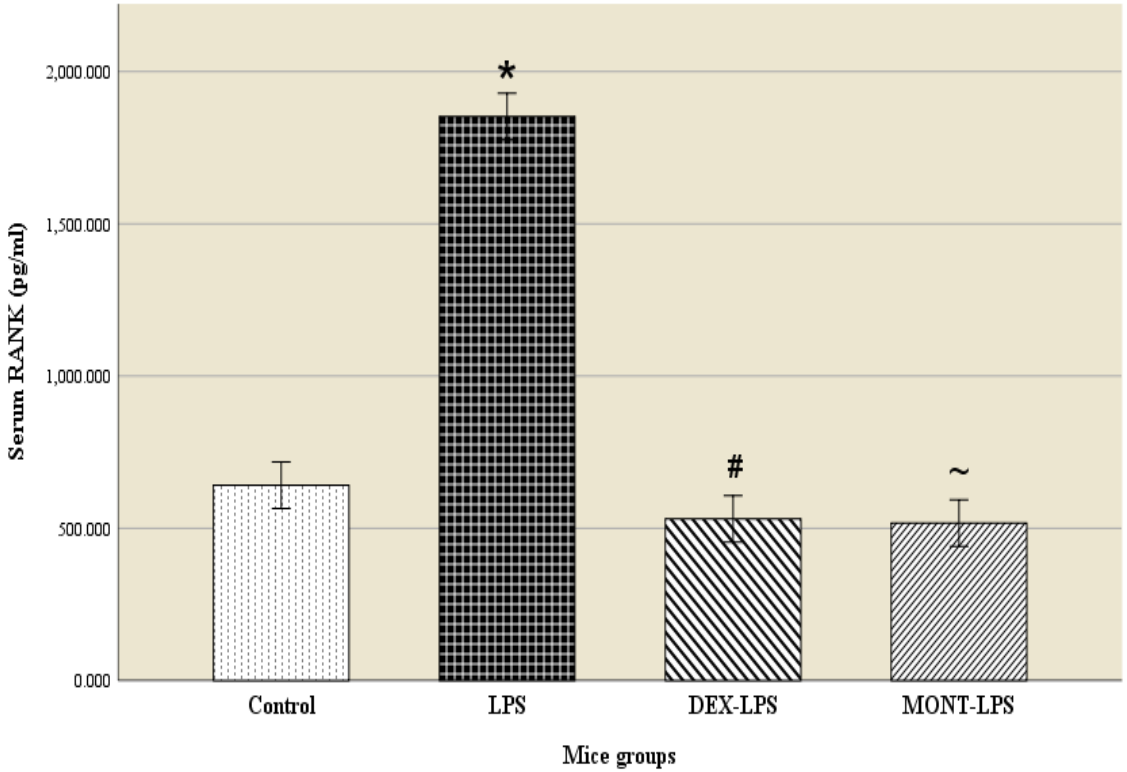
- **Effect of dexamethasone and montelukast on serum TNF-α and IL-6**

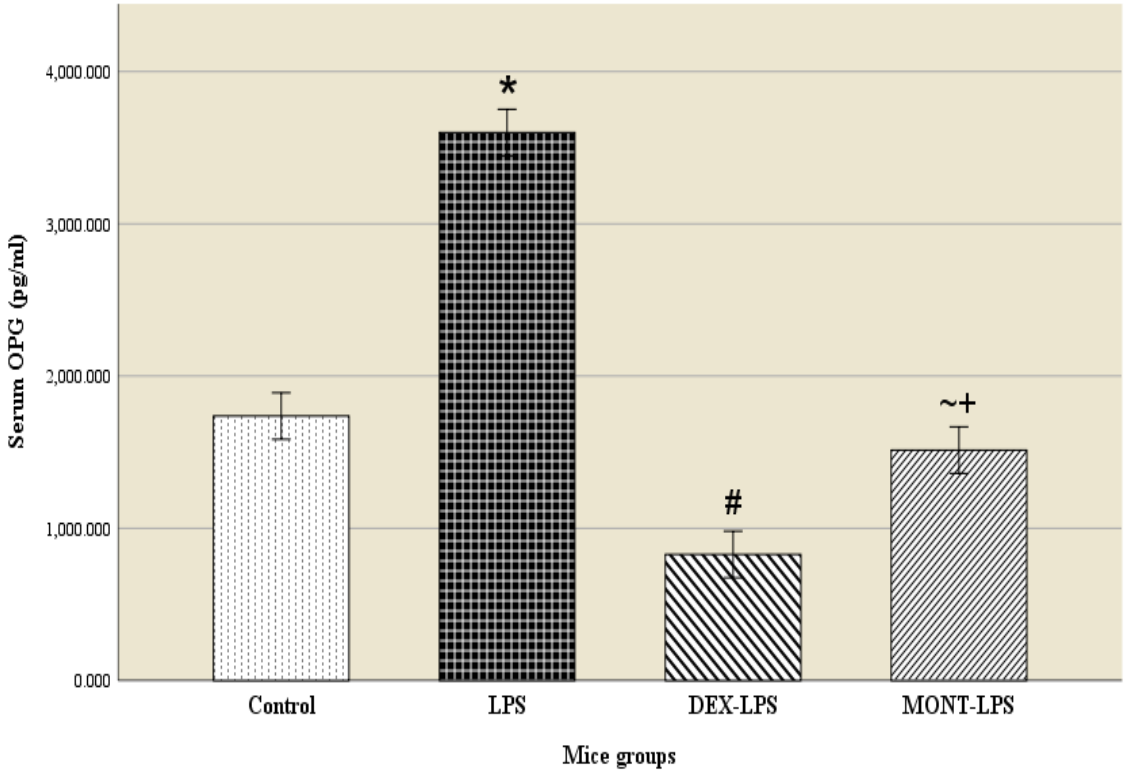




Results

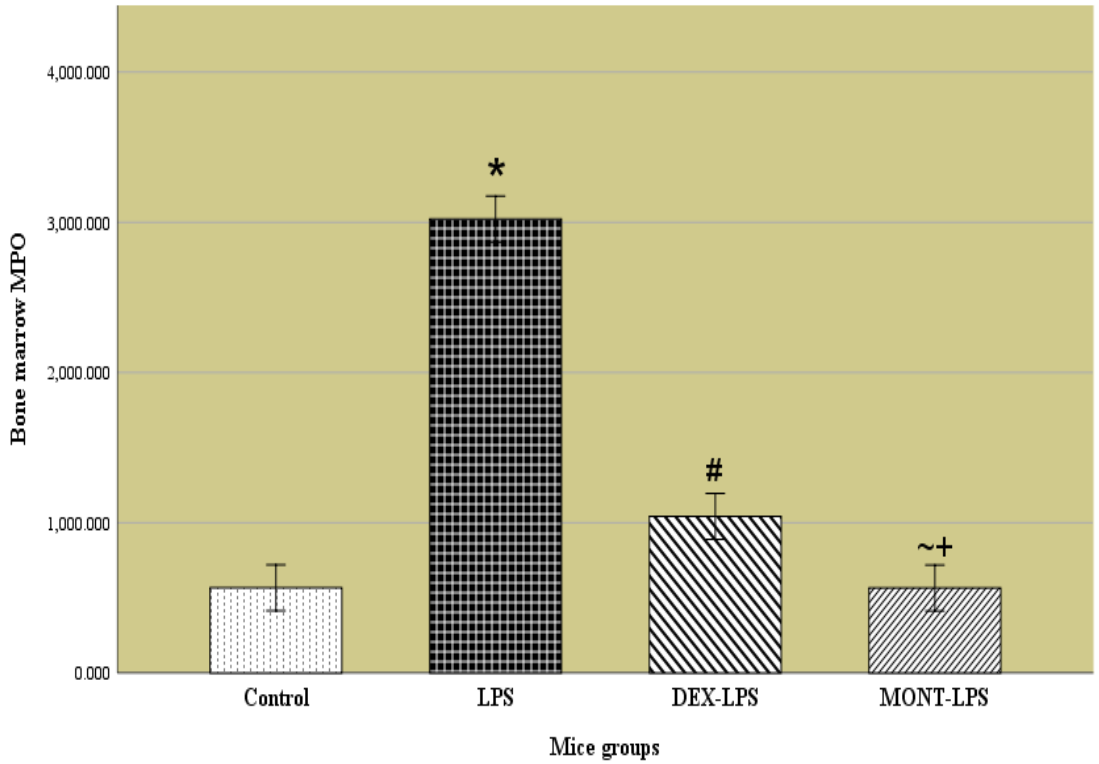
- **Effect of dexamethasone and montelukast on serum RANK and OPG**

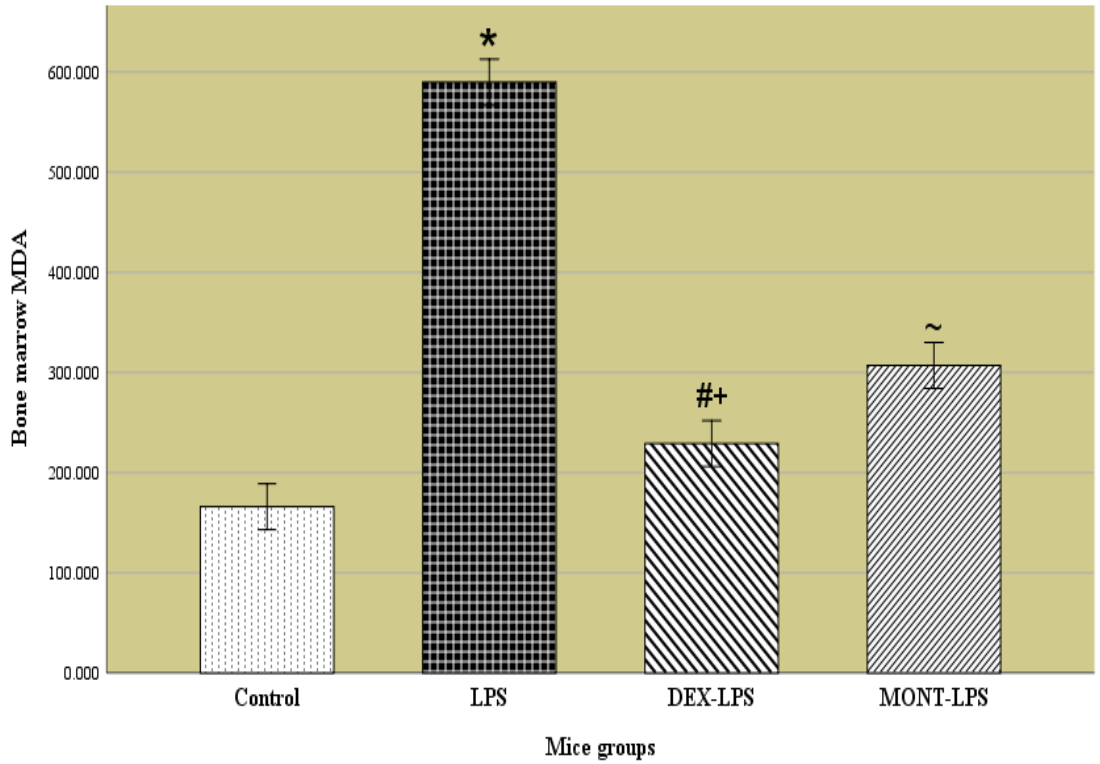




Results

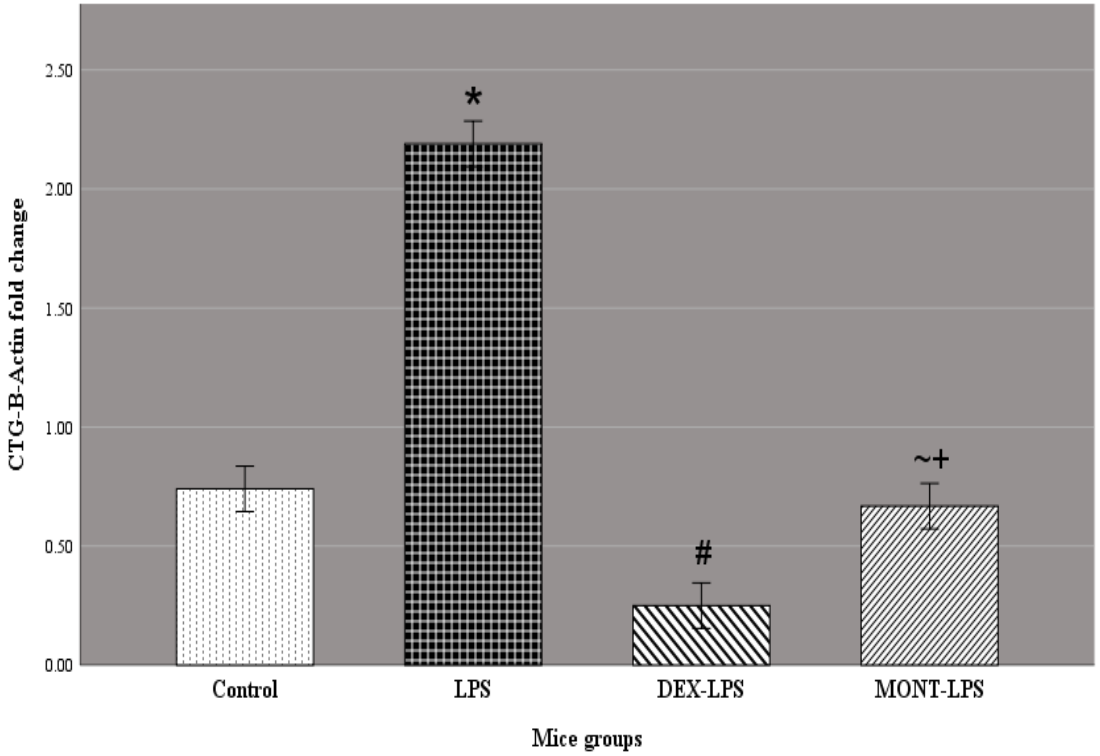
- **Effect of dexamethasone and montelukast on bone marrow MPO and MDA**

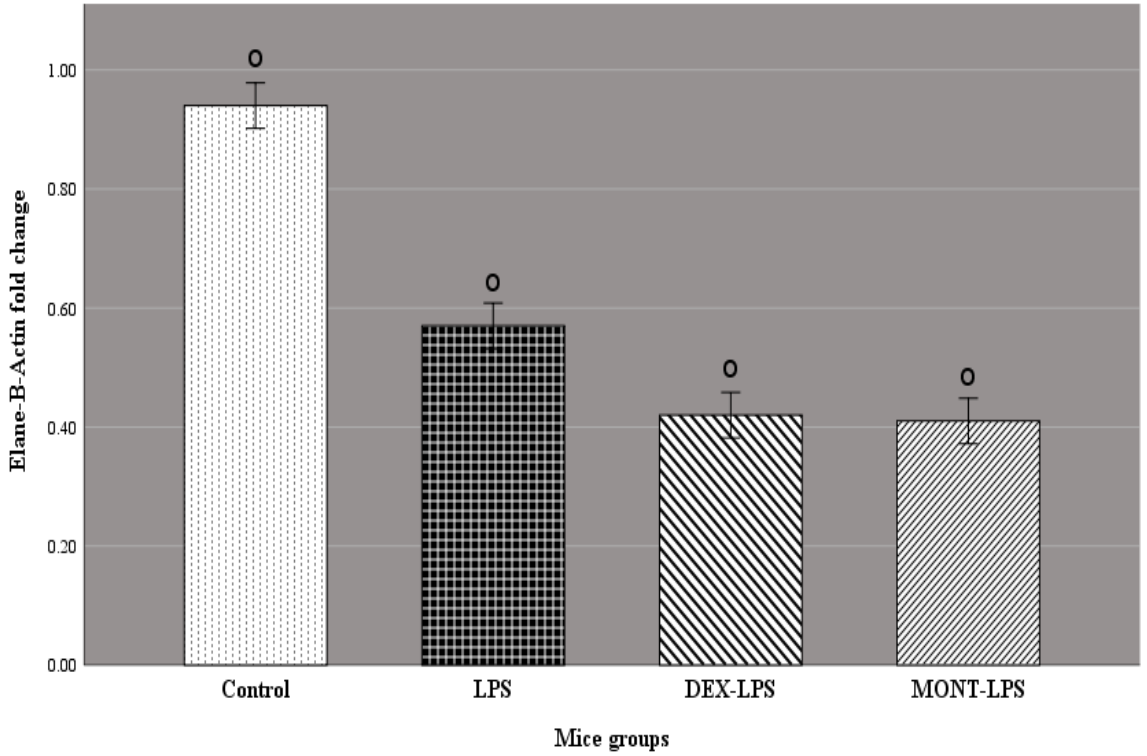




Results

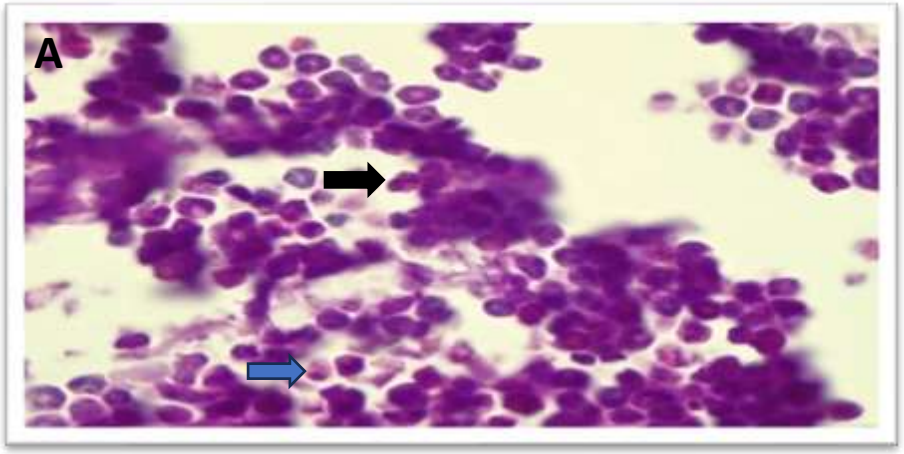
- **Effect of dexamethasone and montelukast on spleen CTG and ELANE genes expression**

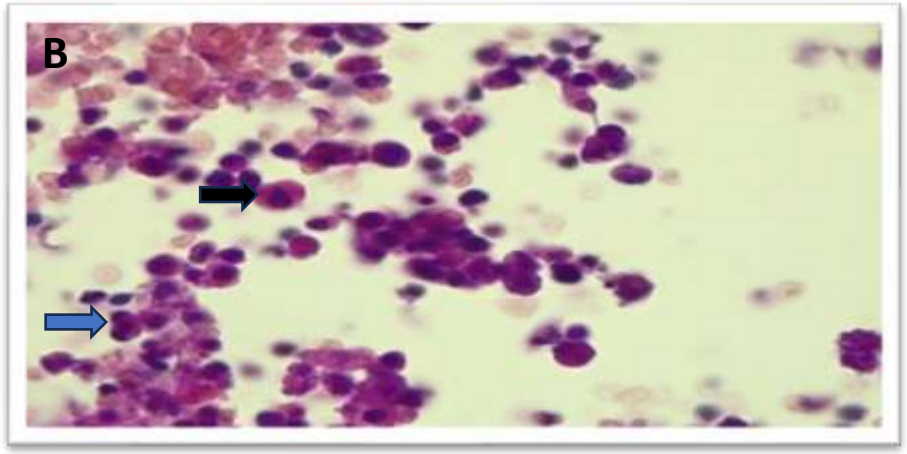


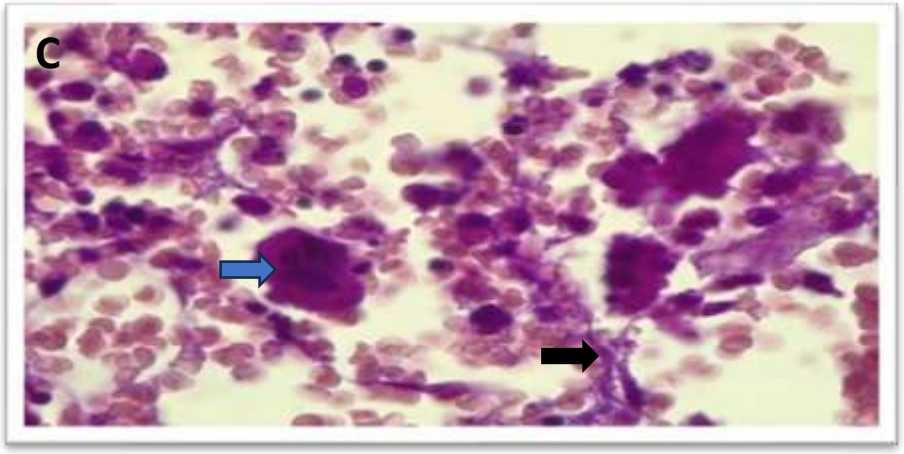


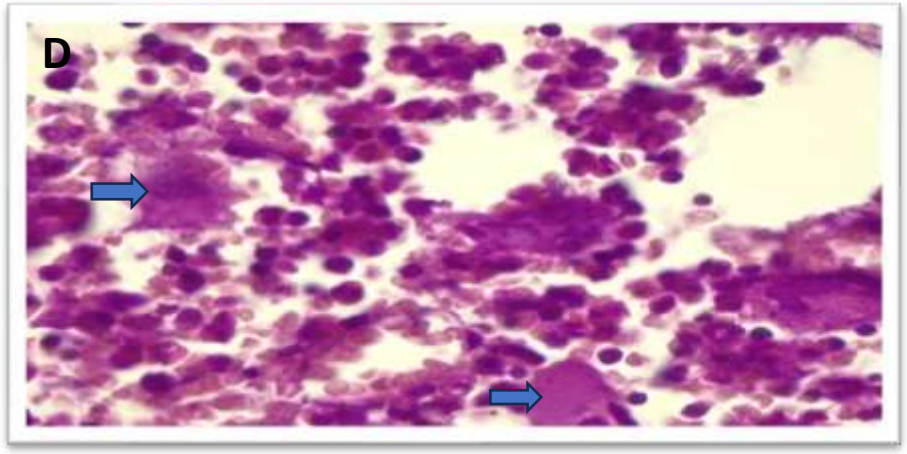
Results

- **Histopathological studies**









Discussion

- Single dose of (IP) LPS significantly increased proinflammatory cytokines such as TNF-α and IL-6, which can activate Toll-like receptors on neutrophils and monocytes, transducing intracellular signals through MYD-88 dependent and independent pathways, leading to activation and translocation of NF-κB, AP-1, and IRF-3 to the nucleus and transcription of many proteins, including proinflammatory cytokines like TNF-α and IL-6, leading to MCP-1 production and phagocytic cell recruitment; This leads to ROS production, which activates pro-inflammatory factors like NF-κB and AP-1, resulting in a vicious cycle of tissue damage.
- Mice pretreated with dexamethasone showed a significant decrease in serum TNF-α levels, even below the control group. Corticosteroids prevent LPS-induced TNF-α release. This antagonism is noncompetitive. Dexamethasone completely blocked TNF production after a short exposure, even when given to cells at the same time as endotoxin.
- Montelukast has a more beneficial effect than dexamethasone, reducing TNF-α release to near-normal values without suppression in this experiment.

Discussion

- Montelukast can reduce IL-6 by reducing eosinophil activity, secretions, and survival, as well as by inhibiting the activity of NF-κB. Besides; Montelukast, as a PPARγ agonist, suppresses NF-κB, resulting in an anti-inflammatory response after LPS exposure in mice.
- Proinflammatory cytokines, including IL-1β, IL-6, and TNF-α promote osteoclastogenesis via RANKL upregulation resulting in bone resorption; Accordingly, anti-inflammatory drugs may prevent bone fractures. Dexamethasone may have some positive effects by their anti-inflammatory effect, they also increase the risk of bone resorption since they enhance RANKL expression and decrease OPG expression.
- This study found raised blood RANK and IL-6 levels, which increased bone resorption risk. However, LPS treatment raised blood OPG levels too. The soluble decoy receptor OPG competes with RANK for RANKL attachment. Data indicates montelukast maintains greater OPG levels than dexamethasone. Montelukast maintains OPG levels closer to the control group

Discussion

- Through proteolytic regulation, NSPs can regulate cell surface chemokines, cytokines, growth factors, and receptors. Neutrophil elastase (NE), a contradictory feature of innate immunity, serves as both a host protection and tissue-damaging agent. NE activity breaks opsonins and phagocytic receptors, preventing neutrophils and macrophages from killing and clearing microbes.
- Neutrophils have at least four types of serine proteases; therefore, NE's function may be obscured or redundant. Low or deficient NE or CTG levels do not change neutrophil recruitment to inflammation, oxidative assault, or phagocytic activity in mice. NE overactivation during early inflammation may subvert the innate immune response, which might worsen the prognosis.
- Dexamethasone's immune-suppressant effects significantly reduced CTG expression levels.
- Montelukast nearly restored normal CTG expression levels that LPS dramatically elevated.

Discussion

- After a single dose of intraperitoneal LPS, MDA, a measure of lipid peroxidation, was significantly raised, indicating that LPS at this dose stimulates oxidative stress (OS) by liberating free radicals that can target membrane phospholipids or polyunsaturated fatty acids (PUFAs).
- As a defensive measure, bone marrow MPO was significantly elevated in the LPS-model group, suggesting that neutrophils and other innate immune cells recruited to the site(s) of injury and released this dual-role weapon MPO to help in microbial killing.
- Dexamethasone reverses the effects of ROS on NF-κB and interrupts the cycle that produces more free radicals. By inhibiting phospholipase A2, dexamethasone and other corticosteroids reduce the release of arachidonic acid, one of the main PUFAs from cell membrane phospholipids, thus decreasing ROS precursors and substrates, lowering lipid peroxidation and MDA.

Discussion

- On the other hand, dexamethasone caused a modest MPO reduction since it did not reach the basal line values of the control group.
- Pretreatment with montelukast caused MDA levels to decrease significantly compared to the LPS model, but they remained higher than control and DEX-LPS groups. However, montelukast significantly reduced MPO levels compared to LPS-model group and DEX-LPS group; this shows its anti-oxidant effectiveness as it normalizes MPO levels (to baseline in control) since there is no significant difference with control.
- A relatively pale slide appearance (LPS group) indicated a marked reduction in hematopoiesis and replacement with fatty tissue.
- Pretreatment with dexamethasone decreased pro-inflammatory cells, and increased fat tissue compared to the LPS-model group and control group. Pretreatment with montelukast showed a decrease in pro-inflammatory cells, with less adiposity compared with the DEX-LPS group.

Conclusions

- Utilizing the findings of this study on albino mice, the following conclusions could be made:

- **1-** Administration of Montelukast prior to LPS can effectively alleviate the acute inflammatory process as evidenced by TNF-α and IL-6 serum levels.

- **2-** Regarding its diverse anti-inflammatory action; Montelukast exerts an anti-resorptive effect on bone as guided by serum RANK and OPG levels.

- **3-** Despite the modest reduction of bone marrow MDA levels; Montelukast exerts a powerful antioxidant effect when given as a pretreatment to LPS-induced OS as demonstrated by bone marrow MPO levels.

Conclusions

- **4-** Montelukast has an immunomodulatory action as proved by normalization of CTG-gene expression in the spleen; Besides, it causes a non-substantial downregulation of ELANE-gene expression.

- **5-** As compared with dexamethasone; Montelukast has a preference in reducing OS, in immune modulation, as anti-resorptive, and comparable anti-inflammatory effect.

Recommendations

- **1-** Investigate the protective effect of montelukast in a polymicrobial sepsis model like CASP.

- **2-** Study the effect of montelukast on other dangerous markers in sepsis such as HMGB-1 and its effect in adaptive immune cells like T-helper types T-regulators and T-suppressors.

- **3-** Trying a combination pretreatment like montelukast plus a macrolide antibiotic or an HMG co-reductase inhibitor as a comparison with montelukast pretreatment alone.

- **4-** Analyze complete blood count CBC with differential count WBCs together with biochemical markers like serum CRP, and lactate.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FOR LISTENING
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Joshua Ray Walker