# Activation of The Immune System by Targeting Macrophage at The Surface Receptors (Myd88) through Covalent Binding TLR4 Antibody onto The Surface of Andrographolide Nanoparticles

**Ariansyah[1], Nurul A.Ade[1], Indah R. Ayu[1], and Hasan Nurhasni[2]**

[1]Undergraduate students, Pharmacy Faculty, Hasanuddin University, Makassar, Indonesia
[2]Faculty member, Pharmacy Faculty, Hasanuddin University, Makassar, Indonesia

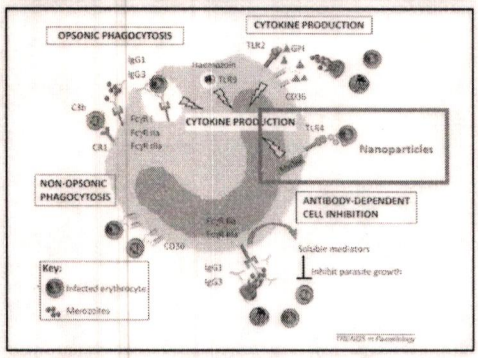
## Introduction

The function of the immunomodulator is to improve the immune system either by means of stimulation (immunostimulatory) or suppress the overactive immune response (immunosuppressants). Some medicinal plants have been studied to have immunomodulatory effects, one of which is Sambiloto (*Andrographis paniculata*). The active component of sambiloto is from the class of terpenoids known as andrographolide isolated from the methanol extract and has been shown to possess immunomodulatory activity and even able to inhibit the induction of HIV disease-causing cells. The Immunomodulatory activity of Andrographolide compound related to macrophage activation and specific antibody response. Andrographolide compound can modulate the innate and adaptive immune responses by regulating macrophage phenotype polarization and Ag-specific antibody production. MAPK and PI3K signaling pathways are also thought to play a role in the mechanism of Andrographolide in regulating macrophage activation and polarization. Macrophages themselves are large white blood cells that have an important function in maintaining the immune system is in charge of keeping the cells and organs of the body from viral infection and bacterial pathogens. So that if macrophages can be activated and becomes the target of the drug delivery system would provide enormous benefits in medicine. Nanoparticles are one of the main parts in the drug delivery system, particle size ranged between 1-400 nm and can serve as a carrier of unstable drug compounds or for the purpose of targeted delivery by modifying the surface of the particles composed of biodegradable and biocompatible polymers. The surface of the nanoparticles can be modified by covalently bound to the monoclonal antibody, apaptamer, and polyethylene glycol (PEG) to a specific target and improve the effectiveness of the active substance. Macrophages themselves possess MyD88 surface receptor which is one of the Toll-like receptor family and is able to bind specifically to TLR4 antibody (HTA125).

## Problem Statement

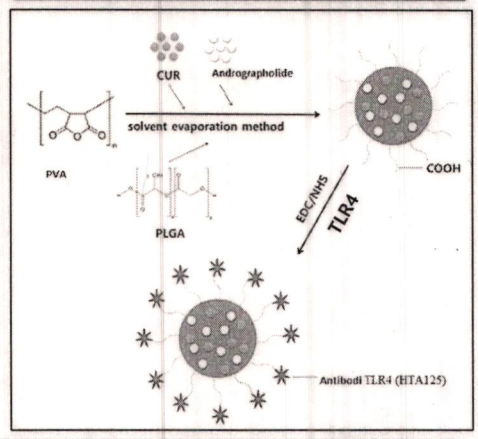
The problem that arises is how to fabricate nanoparticles containing synthesized compounds (Andrographolide) from Sambiloto (*Andrographis paniculata*), which has the best evaluation of nanoparticles for immunomodulatory purposes by way of activating macrophages. Another problem is how to modify the surface of the nanoparticles that can bind to macrophages and provide greater therapeutic benefits.

## Hypothesis

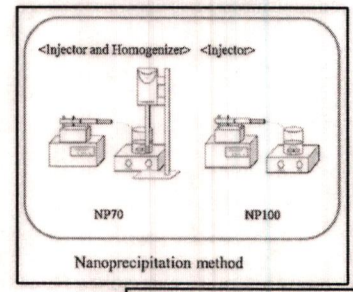


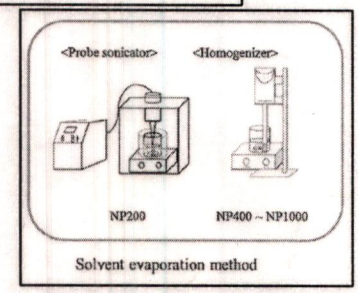

The hypothesis of drug delivery models **(modified from Caroline et all 2012)**

## Methods




## Fabrication methods of size-controlled PLGA nanoparticles

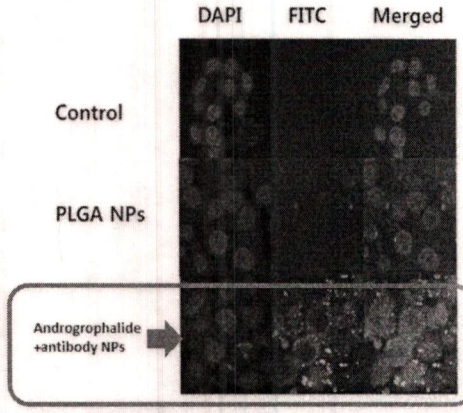



Schematic presentation of fabrication methods of size-controlled PLGA nanoparticles **adapted from Choi, *et.al* (2014)**

## Expected Results

### Cellular uptake



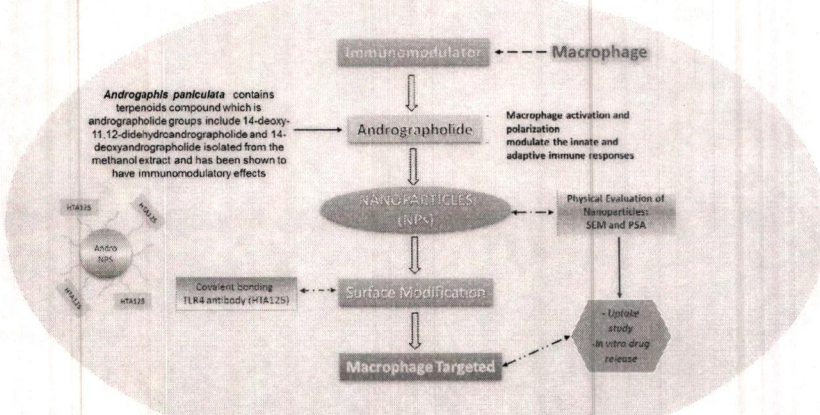

Uptake study of blank NPs and a modified surface NPs **(modified from Choi, et al., 2014)**

## Draft Formula

| Formula | Target Size (nm) | Solvent | PLGA Conc. (Polymer %b/v) | PVA Conc. (Surfactant %b/v) | PVA Volume (ml) | Fabrication method of NPs |
|---|---|---|---|---|---|---|
| I | 70 | Aseton | 1 | 1 | 100 | Nanoprecipitation |
| II | 100 | Aseton | 1 | 1 | 100 | Nanoprecipitation |
| III | 200 | DCM | 1 | 3 | 20 | Solvent evaporation method |
| IV | 400 | DCM | 1 | 3 | 20 | Solvent evaporation method |

The active substance used is 1% w/v and dissolved in a solvent that also dissolves the polymer. The Concentration ratio of active ingredient and polymer is 1:1. PLGA as polymer soluble in organic solvents such as acetone and dichloromethane. PVA is used as a stabilizer and a surfactant. The methods used are nanoprecipitation and emulsification solvent evaporation methods (O/W).

## Conceptual Framework




Conceptual Framework

## Conclusion and Recommendation

♣ Nanoparticles that have been modified by covalent binding to TLR4 antibody (HTA12) is expected to provide a better uptake into macrophages. So that could be a promising approach as novel immunomodulatory agent. This idea has the potential to be developed into actual research.

## Acknowledgments

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## References

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