



**DETERMINATION OF IL-10 EXPRESSION IN
PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs)
TREATED WITH ETHANOLIC EXTRACT OF *GYNURA
PROCUMBENS***

By

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ABSTRACT

Gynura procumbens (*G. procumbens*) is a traditional medicinal herb that commonly used in South-East Asia as food consumption to treat inflammation, skin rashes and viral infection. Interleukin-10 (IL-10) is an anti-inflammatory cytokine act to activate macrophages and B cells, inhibit mast cells and T-helper 1 (Th1) cells, and stimulate T-helper 2 (Th2) cells. This cytokine activated from monocytes, macrophages, and different T-cell subsets which can be mainly found in peripheral blood mononuclear cells (PBMCs). Our aim was to determine the expression of IL-10 in PBMCs treated with *G. Procumbens* aqueous extraction. Peripheral Blood Mononuclear Cells (PBMCs) isolated from whole blood by using Ficoll gradient density were used to determine the activities of *G. Procumbens* on lymphocytes, monocytes and macrophages function to induce the expression of inflammatory cytokines, IL-10 with different concentrations of *G. Procumbens* aqueous extraction (50, 200 and 800 µg/ml). Viability of PBMCs was observed and calculated after staining with Trypan blue and Wright stain. A specific Toll-like receptor (TLR) 4 inhibitor, CLI095 was used to determine whether or not *G. Procumbens* exerts its effects through TLR4. An antagonist of lipopolysaccharides (LPS), polymyxin B, was used to evaluate whether endotoxin properties in *G. Procumbens* contributed to its immunomodulatory activity. Cell culture supernatants were analysed by using Luminex® Human Screening Magnetic Assay. *G. procumbens* exhibited not significantly potent immunomodulatory activity of IL-10 by PBMCs. The expression of IL-10 were increased upon exposure to different concentrations of *G. procumbens* by co-treatment with CLI-095 (TLR4 inhibitor) but partially decreased by polymyxin B (LPS-neutralizer). One-way ANOVA of those treatment groups compared to control group give $p > 0.05$ followed by post-hoc multiple comparison Dunnett's test. We demonstrate impotent immunomodulatory activity of *G. procumbens* towards the expression of IL-10 by PBMCs. We believe that this immunomodulatory activity is due to endotoxin-like properties of *G. procumbens* via TLR4 receptor and MyD88 or Toll/IL-1R domain-containing adaptor inducing IFN- β (TRIF) - signalling pathways.

Keywords: *G. procumbens*, IL-10, inflammation, Luminex®, PBMCs

CHAPTER 1

INTRODUCTION

1.1 Introduction

Herbs or medicinal plants have been used by human since ancient times so much so even the history of herbalism is closely tied with the history of medicine from prehistoric times up until 19th century. Even today many of the primary sources of available drugs have been directly or indirectly derived from plants. For example, Metformin that is widely used to treat and to reduce the occurrence of diabetes was extracted from *Galega officinalis* (Bailey & Day, 2004). In recent year, grounded by a blend of current scientific research and traditional usage, an herbal medicine has become a significant part in the medical healthcare. However, the statistical study also shows there is an improvement in the clinical usage indigenous drugs, which mostly because of their effectiveness and being free from serious toxic effect. Yet, a constant increase in the antibiotics resistant strains and various side effect caused by synthetic drugs have urged scientists and researcher to look for herbal immunomodulator to treat various infections (Fischbach & Walsh, 2009).

Nowadays, medicinal herbs often used as alternative medicine other than of using modern medicine. Medicinal herbs are a good resource of bioactive phytochemicals. Phytochemical is a bioactive non-essential nutrient from plants which may have an encouraging effect on human health. Based on recent study approximately 800 plant species were reported to have anti-diabetic properties and plant-derived compounds are mainly from the group of alkaloids, glycosides, galactomannan gum, polysaccharides, hypoglycin, peptidoglycans, guanidine, steroids, glycopeptides, and terpenoids which have demonstrated bioactivity against hyperglycemia (Patel et al., 2012). According to Han et al., (2007) , the most interesting part of the phytochemical study is phenolic compounds that hold an extensive variety of bioactivities. In the previous study, phenolic compounds are usually known as a contributor of plant chromogen. Now it has been approved that it contains defence mechanism against parasites, pathogen, and other microbes, as well of its importance in plant reproduction and growth (Botia et al., 2001). Based on