# CHAPTER V

# DISCUSSION AND CONCLUSION

Natural products from plants have been used for prevention and treatment of several kinds if illnesses since ancient times. Plants are well known attractive sources of novel therapeutic agents because a tremendous compounds are discovered in millions species of plants. Many natural products or natural derived products are clinically used but there are still enormous natural products remain unexplored. Several groups of compounds have been explored for their potential anti-inflammatory agents especially flavonoids [72-73]. Flavonoids have been reported to exhibit a variety of biological effects both in vitro and in vivo as antibacterial, antiviral, anti-ulcerogenic, cytotoxic, antineoplastic, anti-mutagenic, antihepatotoxic, antihypertensive, hypolipidemic, antiplatelet, antioxidant, and anti-inflammatory activities [5]. *Derris reticulata* Craib is a plant in the Leguminosae family which has been used as expectorant and thirst relief. This plant contains flavonoids as its major active compounds similar to other plants in the genus *Derris*. Lupinifolin has been reported as the major constituent from the stem of *D. reticulata* [3]. Dereticulatin and 2', 3 '-epoxylupinifolin have also been identified from the stems of this plants [74]. Very few pharmacological activities of these flavonoids have been evidenced. Lupinifolin demonstrated inhibitory effect, with out cytotoxicity, on Epstein–Barr virus (EBV) early antigen activation induced by 12-O-tetradecanoylphorbol-13-acetate in Raji cells in vitro and exhibited chemopreventive effect on mouse skin tumor promotion in vivo [66]. Potential anti-inflammatory activity of the ethanolic extract of *D. reticulata* was investigated in this study. This extract at all concentrations did not have any cytotoxic effect on J774A.1 macrophage cells used in the study.

Activated macrophages are the source of several mediators that involve in host defense as well as in inflammatory response. They also increase ability to get rid of pathogens, debris cells and apoptotic cells by phagocytosis. LPS-activated

macrophages produce and release several cytokines as well as pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) [2]. They generate several free radicals such as hydrogen peroxide, super oxide anion and nitric oxide (NO) for intracellular destroying pathogen. These activated cells also produce large amount of prostaglandins $E_2$ ($PGE_2$). All these mediators play different roles in host defense and inflammatory response. However, improper overproduction of these mediators can lead to pathological inflammatory diseases [75].

The ethanolic extract of the stems from *D. reticulata* inhibited NO production in LPS-activated mouse macrophage J774A.1 cells in a concentration-dependent manner with the $IC_{50}$ value at 62.5 μg/ml. Activated macrophages produce large amount of NO from L-arginine by iNOS. The ethanolic extract also inhibited the mRNA expression of iNOS in a concentration dependent manner. These results demonstrate that the extract suppresses the NO production by directly or indirectly down regulating iNOS expression. It has been reported that clinically used anti-inflammatory agents also inhibit of NO production and iNOS expression. Dexamethasone has been shown to inhibit the production of NO and iNOS protein in LPS-stimulated macrophages [76].

Expression of iNOS in activated macrophages is regulated by pro-inflammatory cytokines TNF-α, IL-1 and IL-6. These cytokines up regulate gene expression of several other inflammatory mediators. TNF-α is an endogenous pyrogen which causes fever. It plays an important role in the pathological of inflammation, cachexia and septic shock. TNF-α triggers a cascade of cytokines responsible for attracting macrophages to the site of inflammation and also increases vascular permeability during inflammatory process. IL-1 is also pro-inflammatory cytokine. It is an important component in the initiation and enhancement of inflammatory response to *Helicobacter pylori* infection [77]. IL-6 is a multifunctional cytokine. During inflammation it is one of the important mediators of fever and of the acute phase response. The production of these pro-inflammatory cytokines from activated macrophages is rapidly increased in acute inflammatory responses associated with infection, injury, trauma, and other stressors. Therefore, suppression of these cytokines is a good strategy for the treatment of various

inflammatory diseases. The ethanolic extract significant suppressed the expression of TNF-α, IL-1 and IL-6 genes in LPS-stimulated J774A.1 in a concentration dependent manner. It is possible that the extract down regulate iNOS expression indirectly by suppresses these pro-inflammatory cytokines expression.

LPS activated macrophages also generate excess amount of $PGE_2$ by activation of COX-2 expression [78]. COX-2 is a critical inflammatory enzyme which converts arachidonic acid to prostaglandins (PGs) [52]. It is an inducible enzyme expressed during inflammatory disease in many cells including fibroblasts and macrophages and mediates the release of large quantities of proinflammatory PGs at the site of inflammation causing pain, swelling and stiffness [49]. COX-2 expression in activated macrophages is also regulated by proinflammatory cytokines TNF-α, IL-1 and IL-6. The ethanolic extract of *D. reticulata* suppressed the expression of mRNA COX-2 in LPS-activated J774A.1 cells in a concentration-dependent manner. This result may be the consequence from the decrease in the pro-inflammatory cytokine production of the extract.

It is known that LPS also increases phagocytosis activity of LPS-activated macrophages. These activated cells phagocytose recognized pathogens or foreign particles. They have ability to get rid of pathogens, debris cells and apoptotic cells by phagocytosis. They also generate several free radicals such as hydrogen peroxide, super oxide anion and NO for intracellular destroying pathogens. These substances may be release into the extracellular space and may injure host tissues leading to closely correlated with the pathophysiology of a variety of diseases and inflammation [75]. The ethanolic extract also inhibited phagocytosis activity of LPS-stimulated J774A.1 cells in a concentration-dependent manner.

In conclusion, the results in this study demonstrate that the ethanol extract from the stems of *D. reticulata* suppresses LPS-activated macrophage from expression of several proteins which are pro-inflammatory cytokines, TNF-α, IL-1 and IL-6, and enzymes iNOS and COX-2 that responsible for NO and $PGE_2$ production. This extract did not have effect on cell viability. Therefore, *D. reticulata* may be a source of natural

products which are good candidates for developing novel anti-inflammatory agents in the future.