CHAPTER I

INTRODUCTION

1.1. Background

Malaria is a common and life-threatening disease in many tropical and subtropical areas. Malaria transmission occurs in all six WHO regions with estimated 3.2 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk (> 1 in 1,000 chance of getting malaria in a year). According to the latest estimates, 198 million cases of malaria occurred globally in 2013 (uncertainty range 124–283 million) and the disease led to 584,000 deaths (uncertainty range 367,000 - 755,000). The burden is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occur, and in children under 5 years age, who account for 78% of all deaths[1,2].

Over 1,277 plants belonging to 160 families were reported to be used traditionally for the treatment of malaria following an extensive survey of the literature[3], and since then the number of species has increased substantially due to the increasing worldwide interest in anti-malarial plants. In traditional practice, several plants are often used in combination. Some of them have been screened as crude extracts for *in vitro* and/or *in vivo* anti-plasmodial activity directed to the erythrocytic stage of malaria parasites. Single active anti-plasmodial constituents have been successfully characterized from some extracts, following the pharmaceutical industry paradigm of drug discovery[4-6].

The families plant which stand out due to their broad traditional uses against malaria is *Annonaceae* (ordo Magnoliales). The *Annonaceae* distributed pantropical and comprises approximately 123 genera and 2,100 species[7] of trees, shrubs and lianas. The antiprotozoal activity of *Annonaceae* species in traditional treatments of malaria had been studied[8]. Ethnobotanical reports that *Annonaceous* species is used traditionally to treat malaria and related symptoms (high and intermittent fevers, chills, headaches, body pain, liver ailments) and ethnopharmacological studies demonstrating *in vitro* activity of extracts of plants of this family against *Plasmodium falciparum* (median inhibition concentrations, IC 50 < 1.5 μg/mL) and other malaria parasites.

Annona muricata at the dose of 200 mg/Kg BW/day given before and after *P. berghei* ANKA (PbA) inoculation to the swiss mice as a model for experimental cerebral malaria (ECM) had been studied. Those mice showed a reduced parasitemia level at day 7 (cerebral malaria phase) accompanied by a trend of reduced interferon-gamma (IFN-γ) level produced by spleen cells[9]. In addition those swiss mice also showed significant reduce of tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) level which are both produced by the same cells[10]. Two out of six mice was died in the group of PbA inoculated and treated with 200 mg/Kg BW/day, therefore the doses used in the recent study will be 100 and 150 mg/Kg BW/day.

The studies of CM have independently implicated that cytokines, adhesion molecules, and chemokines have important roles in the disease

pathogenesis. TNF-α and IFN-γ are two proinflammatory cytokines that clearly contribute to CM pathogenesis[11]. Intercellular adhesion molecule-1 (ICAM-1) has long been linked to CM due to its role in parasitized red blood cell (pRBC) engagement and in T-cell adhesion as well as transendothelial cell migration through the brain endothelial cells. Many chemokines have been noted to exhibit increased expression in cerebral malaria, but the chemokines most strongly implicated to have a role are the chemokine (C-X-C motif) receptor 3 (CXCR3) binding chemokines, including CXCL9, and CXCL10[12-14]. The CXCL10 is the first known chemokines which is able to direct the trafficking of activated effector CD4+ and CD8+ T lymphocytes, beside natural killer (NK) and natural killer T (NKT) cells. CXCL10 the binding of high-affinity receptor CXCR3, are also capable of binding CXCL9 and CXCL11[15]. The importance of CXCL10 had been suggested by several studies in malaria.

A study of CM in Ghanaian children demonstrated that out of 36 biomarkers, only CXCL10 was serum marker independently associated with CM mortality[16]. In addition, study of cerebral spinal fluid (CSF) of CM patients showed that CXCL10 was one of eight biomarkers significantly up regulated in the CM group. The ECM study showed that CXCL10 was highly induced in the brain especially at the neuron of *P. berghei* ANKA infected mice, and CXCL10 deficient mice were protected partially from CM mortality[12]. The recent study showed that CXCL10 provoked the apoptosis of both human brain microvascular endothelial cells and neuroglia cells *in vitro*[17].

It is a hope that *Annona muricata* inhibit CXCL10 and capable reducing the inflammation.

1.2. Research Questions

The major research question in this study:

Can *Annona muricata* decrease CXCL10 in the brain of Swiss albino mice inoculated with *Plasmodium berghei* ANKA?

1.3. Research Objective

A. muricata may decrease the CXCL10 in PbA- inoculated swiss albino mice.

1.4. Research Benefits:

This study will give the information about the effects of *A. muricata* toward CXCL10 expression in the brain during CM phase.

1. Clinical application field: The information will give insight for CM management.
2. Science: The information will add the explanation about the mechanism used by *A. muricata* implicated in CM.
3. Research: The information will open future study which confirm the findings and add detail mechanisms involved.

1.5. Research Originality

Table1: Previous report related to study

No	Title publication and authors	Method	Results
1	Beta Interferon Suppresses the Development of Experimental Cerebral Malaria (Craig, et. al., 2011)	The mice were infected with *Plasmodium berghei* ANKA by intraperitoneal (i.p.) injection of approximately 0.5-106 parasites. The mice were treated with IFN-β (800,000 U/mouse/day) by the i.p. route. ecombinant murine IFN-β was obtained from PBL Interferon Source, Inc.	Modified by IFN-β administration. P. *berghei* infection resulted in increased expression of chemokine (C-X-C motif) ligand 9 (CXCL9) in brain vascular endothelial cells that attract T cells to the brain, as well as increased T-cell chemokine (C-X-C motif) receptor 3 (CXCR3) expression. The infection also increased the cellular content of intercellular adhesion molecule 1 (ICAM-1), a molecule important for attachment of parasitized RBCs to the endothelial cell. IFN-β treatment leads to reduction of CXCL9 and ICAM-1 in the brain, reduction of T-cell CXCR3 expression, and downregulation of serum tumor necrosis factor alpha (TNF-α).
2	Chemokines reseptor CXCR3 and its Ligands CXCL9 and CXCL10 are Required for the Development of Murine Cerebral Malaria (Gabriele, et. al., 2008)	The mice infected by chemokines in the development of cerebral malaria, and the IFN-inducible CXCR3 chemokine ligand IP-10 (CXCL10) was recently found to be the only serum biomarker that predicted cerebral	The CXCR3 chemokine ligands CXCL10 and CXCL9 were highly induced in the brains of ECM mice inoculated by PbA. CXCR3-deficient mice were obviously protected against ECM along with extremely fewer T cell number in the

No	Title publication and authors	Method	Results
		malaria mortality in Ghanaian children.	brain compared with wild-type mice.

CXCL 10 and its receptor (CXCR3) studies had been done in ECM. However CXCL10 during cerebral phase has not been studied in *A. muricata* treated mice before and after PbA inoculated swiss mice.