Chapter

Malarial Pathophysiology and Phytochemical Interventions: A Current Discourse on Oxidative Stress Anti-Disease Phytotherapeutics

Greanious Alfred Mavondo, Blessing Nkazimulo Mkhwanazi, Joy Mavondo, Wisdom Peresuh and Obadiah Moyo

Abstract

Malarial systemic pathophysiology refers to physiological changes or abnormalities that are experienced by individuals infected with the *Plasmodium* parasite not be presenting in the absence of active, chronic or previous infection. The pathologies are derived, in part, from OS induced insults whose mediators are readily available in malaria. The malaria disease is equivalent to the pathophysiology as shown by the abnormal syndromic expressions ranging from ailments that affect homeostatic mechanisms and processes to tissues and organ specific damages and derangements. Phytotherapeutic remedies refer to the natural phytochemicals or plant medicinal compounds and their derivatives with known antiparasitic and antimalarial disease effects in both experimental and clinical situations. The chapter explores how *Plasmodium* infection generates or cause to be generated oxidative stress, how oxidative stress drives systemic disease process and how phytotherapeutics treatment (artemisinins) and administration (asiatic acid) in malaria resolves the various pathologies as a current situational analysis.

Keywords: malaria, phytochemicals, phytotherapeutics, asiatic acid, artemisinin, severe malaria anemia, *Plasmodium falciparum*, oxidative stress, reactive oxygen species, anti-disease

1. Introduction

Inferences into free radicals' release and their subsequent OS generation has been described as the causes and drivers of malaria [1–3]. The *Plasmodium* [4]-free radical production [5]-antioxidant defense systems [6] triumvirate axis may be elaborate in the host cell as a pathologic apparatus triggered to subside malarial infection intensity. The character of OS has scarcer shades of clarity up to date with some authors insinuating a protective facilitation against malaria disease, others claim a pathophysiologic role in the pathogenesis of the disease [6]. Studies,

however, tend to associate the production of reactive oxygen (ROS) and nitrogenous species (RNS) with OS (OS) in the development of complex sequalae and systemic malarial disease and its outcomes.

Malarial parasite infection invokes hydroxyl free radical ($OH^{\cdot}$) production by the hepatocytes which may induce OS and apoptosis of liver parenchymal cells [7]. Additionally, it has been observed that parasitized red blood cells (pRBCs) generate $OH^{\cdot}$ radicals and H_2O_2 at approximately double the concentration found in non-parasitized red blood cells (npRBCs); an elicitation from the abundant intracell and endogenous redox reaction players.

Free hemoglobin (Hb), copiously available in malaria, is also a readily obtainable foundation of free radicals as the *Plasmodium* parasite uses the Fe^{2+}-containing molecule as a fountain of amino acids crucial for its sustenance during the erythrocytic stage of disease. The main component and source of protein-bound and free Fe^{2+} is high levels of haeme. The haeme-Fe^{2+} complex induces intravascular OS with deleterious conformational changes to the red blood cells (RBC's) and the endothelial cells. Consequentially, release from pRBC's during haemolysis and subsequent internalization of malarial parasites into liver and brain tissues ensues with varied malarial syndromes presentations [8].

Generally, free haem release during cell haemolysis has a prospective capacity of increasing OS through the Fenton-Fritz Haber-Joseph Joshua Weiss reaction [9, 10] which iron catalyzes, mainly: $Fe^{3+} + {}^{\cdot}O_2^{-} \rightarrow Fe^{2+} + O_2$.

The second step is the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + {}^{\cdot}OH$.

Net reaction: ${}^{\cdot}O_2^{-} + H_2O_2 \rightarrow {}^{\cdot}OH + OH^{-} + O_2$.

And OS created by excess hydroxyl radicals (${}^{\cdot}OH$), superoxide ($O_2^{\cdot -}$), reactive non-radical compounds including singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), lipid hydroperoxides, hypochlorous acid (HOCl), chloramines (RNHCl), and ozone (O_3) [11, 12] potentiates malarial infection patency and snowballing risk for fulminant disease. In the non-immune individuals, cell-mediated immune response is the initial defense mechanism which generates OS as well and subsequently, aberrant immune system response with disease traction increases.

Reactive nitrogen radical compounds such as nitric oxide (${}^{\cdot}NO$), nitrogen dioxide (${}^{\cdot}NO_2$), and non-radical nitrogen-based compounds which include peroxynitrite ($ONOO^{-}$) and dinitrogen trioxide (N_2O_3), make up the collective group of reactive nitrogen species (RNS). The unpaired electrons in their outer electron obit make these species very unstable and highly reactive. Reactive nitrogen species have direct linkages to ROS, especially in the formation of $ONOO^{-}$ which gives rise to nitrosative stress (NS).

The combination of OS and NS have been associated with the etiology of an extensive variety of disease processes and states to include aging, infections, ischemia-reperfusion (I/R) injury [13], acute kidney injury (AKI) and chronic kidney diseases [14], diabetic neuropathies [15], inflammatory disease [16], vascular dysfunction and hypertension [17], atherosclerosis, neurological diseases [18] including Alzheimer's disease [19, 20]. Most of these conditions and diseases are displayed as syndromes and facets of malaria disease.

Management of malaria, will of necessity therefore, require the inclusion of anti-disease remedies that will concurrently suppress or eradicate the pathophysiology associated with malarial. There have been strides to invent remedial treatment for malaria by improving the potency of current antimalarials. However, this avenue does not correspond to requirement of ameliorating the disease aspect of malaria. Phytochemicals, some in basic research stage of investigation or clinical stages, show promising outcomes that are worth promulgating, formulating and pre-empting strides towards attempts to eradicate both the parasite and the sequalae of malaria.

1.1 Malaria causes

There are five main Phylum Apicomplexa (Sporozoa) *Plasmodium* strains inflicting human malarial infection with changing disease outcomes, mainly *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and a zoonotic parasite *P. knowlesi*. Accordingly, parasite species, sub-species, host genetics and host demographics at point of infection, determine malaria disease presentations of varying intensities [21]. A disease process and time-lines with differing syndromic mediators is generated [22, 23]. The *P. falciparum* infection has the highest fatalities with strains having developed multidrug resistance. Artemisinin derivatives are the latest additions to the rug-casualties list. *P. vivax* and *P. ovale* present chronic disease with quiescent liver stage parasite hypnozoites driving disease relapses onwards of 7 years duration from initial infection reported [24]. However, there is divergent of opinion straying from this recrudescence dogma [25].

1.2 Phenotypic presentations of malaria

Malaria disease displays manifestations in adults from non-endemic areas as a different disease phenotype when compared to pregnant women and to children under the age of 5-years. Accordingly, the pathophysiology of malaria, or malaria disease [26] displays immunological idiosyncrasies, inflammatory aberrations, haemolysis which may lead to severe malaria anemia (SMA) [27], acute kidney injury (AKI) [28], and malaria cachexia leading to cardiac failure [29], hypoglycaemia [30, 31], acute respiratory distress syndrome (ARDS) [32], acute lung injury (ALI) [33], cerebral malaria [34], hyperlactaemia with non-respiratory acidosis. Of these pathophysiology, children invariably develop SMA [35], hypoglycaemia [36], hyperlactaemia with non-respiratory acidosis and cerebral malaria while adults presents with severe malaria, AKI [37] and non-respiratory acidosis [38]. Pregnant women present with placental malaria with SMA also a common feature [39]. The bottom line to all these manifestations is the OS mediation to the disease process driven by various species emanating from the parasite-human host interactions. The perceived relationship between the antioxidant capacities displayed by phytochemicals and phytotherapeutics and the oxidant-driven malaria disease motivates this chapter.

2. Malarial systemic disease and phytochemicals administration

The terms phytotherapeutics, phytotherapeutics are commonly used in the branch of science involved in the use of plant natural products and their derivatives and their use as disease management alternatives and ameliorates. The discovery that phytochemicals like the artemisinin, asiatic acid (AA), oleanolic acid and masilinic acid (MA) have both anti-inflammatory (and other physiological influences) activity and antimalarial activities have led to the exploration of their anti-disease action in malaria.

3. Oxidative stress and artemisinin malarial treatment

Artemisinin is a tetracyclic 1,2,4-trioxane containing an endoperoxide bridge (C—O—O—C), the key pharmacophore of the antimalarial [40] (**Figure 1**). Increasing solubility and pharmacological of the drug has been achieved when semi-synthetic compounds were synthesized through modification of C10 in the

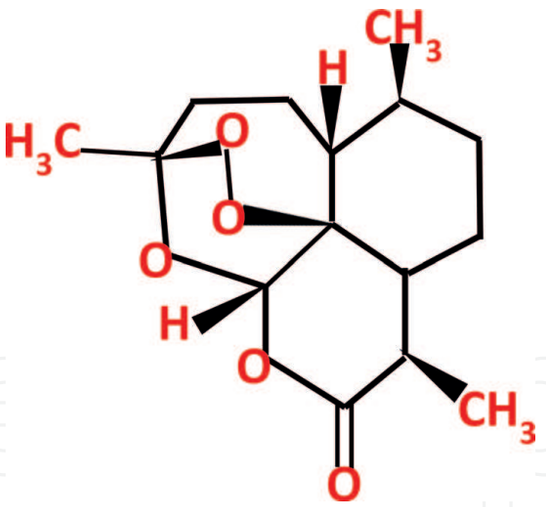

Figure 1.
Artemisinin, structure of the endoperoxides [42].

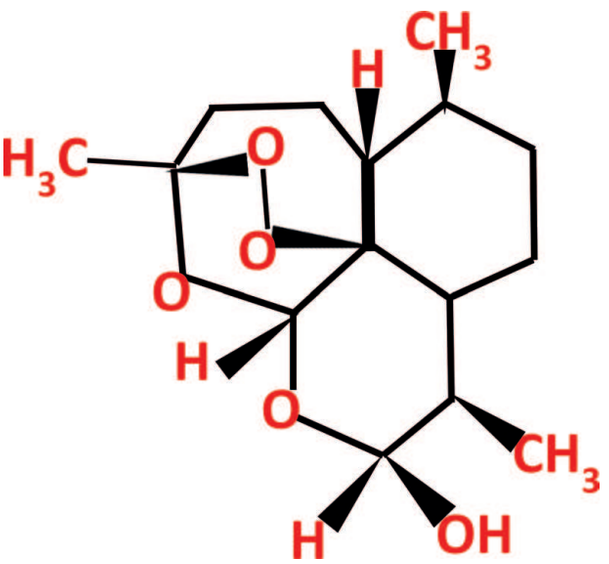

Figure 2.
Dihydroartemisinin, structure of the endoperoxides [42].

original backbone to generate hemi-acetal or ester derivative such as dihydroartemisinin (**Figure 2**), artemether (**Figure 3**) and artesunate (**Figure 4**) [41].

The debate remains unresolved on the mode(s) of activation and consequent biological target(s) of endoperoxides [43]. Activation of the endoperoxide bridge is believed to be the source of artemisinin antimalarial activity. Cleavage of the bridge, which is located at the core of the structure, generates short-lived cytotoxic oxyradicals in the presence of haem iron or free iron Fe^{2+} [44, 45]. However, two different mechanisms of action premised on the endoperoxide bioactivation, have been proposed.

Rearrangement of the oxygen-centred radicals, to produce more stable carbon-centred radicals, have been hypothesized by the Poster Laboratory using 18O-labeled trioxane analogues [46, 47]. The 'reductive scission' model, has ferrous iron binding to either O1 or O2 cleaving the endoperoxide bond and generating oxyradical intermediates which subsequently rearrange to primary or secondary carbon-centred radicals via either b-scission or a [1,5]-H shift. This hypothesis has been supported through evidence of the formation of these carbon-centred radical intermediates using electron paramagnetic resonance spin-trapping techniques [48, 49].

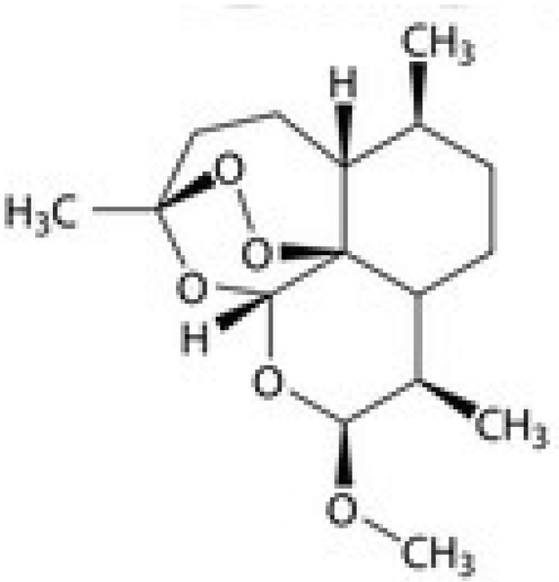

Figure 3.
Artemether, structure of the endoperoxides [42].

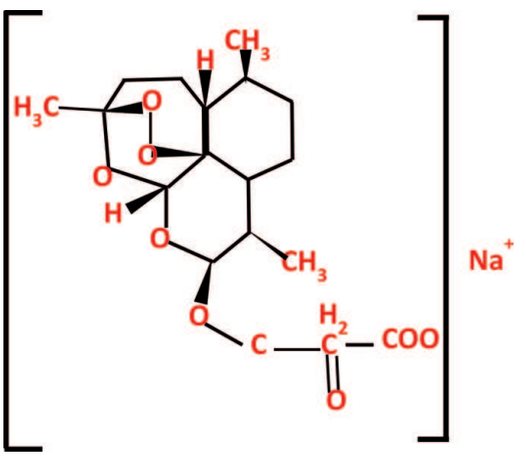

Figure 4.
Artesunate, structure of the endoperoxides [42].

Capabilities of the C-centred radicals to drive haem and or proteins alkylation have been proposed. However, the only evidence, so far provided, has been for haem alkylation [50] and a few reported model studies on protein alkylation with ferrous salts reactions in the presence of cysteine (iron-sulfur chelates) [51].

The concept of free radical generation and protein alkylation points towards creation of OS as the parasite killing apparatus of the artemisinin derivatives but also indicates the deleterious effect of the drugs on the human host and possibility of initiating or exacerbating malarial disease in the course antiparasitic activity. However, paradoxically artemisinin has been reported to be pluripotent with anti-inflammatory activity [52] although post treatment artemisinin haemolysis has also been observed in people that would have been cleared of malaria. The latter adverse reaction has been seen several weeks after successful treatment with artemisinin drugs.

3.1 Artemisinin and anti-oxidative stress disease inductions

Artemisinin and its derivatives require conversion to the biologically active dihydroartemisinin (DHA **Figure 2**) to exert their activity. Besides the excellent antimalarial effects, there is clinical and experimental evidence that suggests potent

immune-suppressive against autoimmune and allergic disease of artemisinin and its derivatives [52]. Derivatives of artemisinin possessing lower range toxicity, higher bioavailability, and compelling immunosuppressive activity have been studied and some commercialized. These are 3-(12-β-artemisininoxy) phenoxyl succinic acid (SM735) [53], 1-(12-β-dihydroartemisinoxy)-2-hydroxy-3-tert-butylaminopropane maleate (SM905) [54, 55], ethyl 2-[4-(12-β-artemisininoxy)] phenoxylpropionate (SM933) [56], and 2′-aminoarteether (β)maleate (SM934) [57, 58].

To date, it is generally accepted that artemisinin wields antimalarial properties through (i) haeme or free iron breaking the peroxide bridge resulting in the degradation of artemisinin molecular structure to form the nucleophilic radical metabolite with the centre at C4. (ii) subsequently, the free radical, acting as an alkylating agent, will attack macromolecular bearing electrophilic groups or centres, which ultimately leads to parasitic demise [50, 59]. In fact, the pRBC's have increased concentrations of OS due to the parasitic infection by *Plasmodium*. In the intervening time, OS driven free radicals and lipid peroxidation concentrations dramatically increase intracellularly. Apparently, pRBC's are rendered more susceptible to artemisinin than npRBC's. In vivo artemisinin is effective in killing pRBC's at nM concentrations which differs sharply when contrasted to marginal effects of artemisinin on resting RBC's even at high mM concentrations [60, 61].

3.2 Anti-inflammation and immunoregulatory effect of artemisinin

There are three fundamental steps by which T cells perform pivotal role in acquired immune reaction [62, 63]: (i) G0 to G1 transition of T cells is driven by TCR cross-linking which leads to the secretion of T cell growth factor IL-2 and expression of high-affinity receptor IL-2Rα chain (CD25); (ii) through autocrine and or paracrine proliferative loop, IL-2 influences clone expansion and maintains activated T cells survival; (iii) after efficacious clearance of the pathogen, the inducement for cytokines production is lost and activated T cells will undergo apoptosis.

Nevertheless, in autoimmune diseases, due to the tenacity of autoantigen, autoreactive T cells will be activated and with better survival [52]. Autoreactive T cell proliferation is involved in the pathogenesis of various immune-related diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS) [20, 21] as well as malaria.

Artemether is a powerful antimalarial drug [64] found to significantly suppress the proliferation and synthesis of IL-2 and interferon-γ (IFN-γ) by T cells through the TCR engagement influence [65]. The TCR engagement-triggered MAPKs signaling pathway as well as phosphorylation of ERK1/2, Jnk, and P38 were significantly inhibited by artemether. Discovery was made that artemether greatly affects T cells function as compared to that of the antigen presenting cells (APCs) to exert the immunosuppressive effects [65].

A series of artemisinin derivatives, with higher water solubility and lower toxicity, have been created by inserting, to the parent artemisinin structure, new functional groups like ethylene glycol [66, 67]. These have immunosuppressive targeted at T cell activation suppression, combat inflammation through substantial inhibition of the proliferation and production of IFN-γ, IL-12 and IL-6. While there is no direct influence of artemether and its derivatives on IL-2 and CD25 upregulation of T cells, there is remarkable suppression of IL-2-mediated proliferation and survival of activated T cells alluding to blocking of IL-2 induced phosphorylation of Akt [68]. Additionally, artemether derivative SM934-driven preferential early apoptosis activated of T cells, with no effect on resting T cells, has been observed through staining of CD69 and annexin V.

Furthermore, studies suggesting that artemisinin derivatives bind to calmodulin to inhibit phosphodiesterase activity, which causes the increase of intracellular cAMP concentration, and therefore to exert the immunosuppressive activity have been reported [69, 70].

Additionally, oral treatment of SM905 has been shown to skew the T cell subset from pathogenic Th17 to protective Th2 subset in an arthritic model with increased IL-4 production and suppression of the RORγt mRNA expression together with IL-17 production [71]. The artemisinin derivatives' effects hinge on the anti-inflammatory properties which play an antimalarial role. To back this assertion up, artesunate has minor effects on inflammatory responses downstream of antibody production demonstrating that highly proliferative germinal centre B cells are the most sensitive cellular targets to the treatment. Significantly, *in vitro* artesunate inhibits IL-1β, IL-6, and IL-8 production by way of stimulation by TNF-α as well as the expression of vascular endothelial growth factor and hypoxia-inducible factor-1α [72]. Moreover, artesunate inhibits Akt phosphorylation and IκB degradation by blocking PI3K/Akt signaling pathway downstream of TNF-α [73] making it an efficient adjunct to malarial inflammatory response characterized by increased and decreased Th1 and Th2 type cytokines, respectively.

The intriguing anti-inflammatory properties of the artemisinin (SM933) are observed through the regulatory mechanism involving the NF_KB and Rig-G/JAB1 pathways which regulation alters cell cycle activity of activated T cells selectively. In contrast to SM933, SM934 and DHA treatment is majorly through the regulation of the balance between effector T cells and regulatory T cells. Administering of DHA significantly decreases effectors CD4 T cells and increases in Treg cells in a reciprocal regulatory process through modulation of the mTOR pathway which characterizes its regulatory mechanism [56, 74].

3.3 Artemisinin structure: activity relationship in oxidative stress

Macromolecules bearing electrophilic groups or centres are prone to alkylating nucleophilic radicals, metabolites of artemisinin, which eventually leads to cell damages [61]. Furthermore, artemisinin inhibits endoplasmic reticulum Ca^{2+} ATPase (SERCA) with consequent cytoplasmic calcium accumulation and secondary activation of cellular calcium influx. In this way artemisinin induces *P. falciparum* cellular apoptosis [75]. The same effect has been shown by thapsigargin (TG), a specific SERCA inhibitor with structural similarity to artemisinin, which induces cellular calcium accumulation leading to apoptosis. While artemisinin and TG have the same binding sites on SERCA, there are structural biology differences in the binding pocket for different mammalian and *Plasmodium* species resultantly conferring differential susceptibility to the drug. A single amino acid (Leu263) in the transmembrane segment 3 of SERCA in *P. vivax* SERCA (PvCERCA) has a 3-fold sensitivity to artemisinin while introduction of the same residue in *P. berghei* SERCA (PbSERCA) decreases sensitivity 3-fold [75, 76].

Interestingly, while the peroxide bridge plays a necessary role in the artemisinin biological activity, artemisinin-SERCA binding does not involve this moiety [77] but play a catalytic role in the inhibition [52]. Naturally, stereochemistry and transitional state theory have it that the intact peroxide bridge makes the spatial configuration of artemisinin to be relatively rigid and making the sesquiterpene lactone unable to flexibly rotate and fold. This results in lower affinity for SERCA. Reduction and breaking of the peroxide bridge by divalent iron ion, however, releases the sesquiterpene lactone increasing its flexibility and binding affinity to SERCA enhancing inhibitory effect of artemisinin on the enzyme. Moreover, the concentration of Fe^{2+} in pRBC's and activated lymphocytes is significantly increased

compared to resting state cells increasing the opportunity for peroxide bridge to be broken. In this manner, the pRBC's and activated leukocytes, rather than npRBC's and resting cells, become more vulnerable to artemisinin activity. Nevertheless, the mammalian SERCA is not susceptible to artemisinin inhibition [75]. Therefore, the biochemical mechanism and artemisinin molecular target to exert immunosuppression in malaria and other disease driven by OS requires further perusal and investigations.

4. Asiatic acid (AA) administration and oxidative stress-driven malarial disease

Asiatic acid (AA), in **Figure 2**, is a naturally occurring pentacyclic triterpenoid originating from the herb *Centella asiatica*. Triterpenoid saponins are the primary constituents of *C. asiatica* responsible for extensive therapeutic actions. The molecular mechanisms underlying the various biological activities of AA have been described for malarial diseases as it is driven by OS. The pharmacological properties of AA and its derivatives inhibit multiple pathways of intracellular signaling molecules and transcription factors that are involved in the various stages of acute and chronic diseases [78]. The anti-inflammatory [79], anti-hyperlipidemia, malarial hypoglycaemia ameliorative effective [30], neuroprotective, reno-protective effects [80] and other activities of AA have an intricate and elaborate pattern, amongst the triterpenes, that is envisaged on the pleiotropic characteristics bed rocked on its anti-oxidative pro-oxidative properties and redox reactivity [81, 82].

4.1 Severe malaria anemia (SMA) and anti-disease effects of asiatic acid activity

One of the major causes of morbidity and mortality in malaria is SMA driven by a multifaceted etiology. Noteworthy mechanisms contributing to SMA include (i) increased destruction of pRBC's and npRBC's (immune system mediated haemolysis, phagocytosis, splenic sequestration); (ii) decreased RBC's synthesis from immune system and parasite effects [83, 84]. The number of RBC's turnover is a balanced process under normal physiology and a decrease in concentration (anemia) is predicted by an efflux of reticulocytes from hematopoietic tissues. When anemia develops from increased RBC's destruction, such as haemolysis or hemorrhage, erythropoietin (EPO) production in kidneys is normally up-regulated with a concomitant increase in reticulocytosis to alleviate rheological disturbances. Together with EPO, growth factors and cytokines, to include granulocyte colony-stimulating factor (G-SF), stem cell factor (SF), insulin-like growth fator-1 (IGF-1), take active involvement in erythropoiesis [85, 86].

Therefore, production of EPO in the peritubular fibroblasts of the renal cortex, requires that the kidneys physiology be normal [87, 88] and is determined by the amount of oxygen present which depends on the concentration of RBC's. Tissue oxygen tension and hypoxia regulates EPO production in a feed-back loop with hematocrit, centred on an inverse logarithmic relationship [89]. Increased hypoxia resulting from SMA causes increased OS. Therefore, antioxidant capacity of AA is able to correct hematocrit and ameliorating SMA [26]. However, the principle by which this effect of AA on OS in SMA is premised on its effect in reducing kidney lipid peroxidation and increasing antioxidant defenses in malaria.

This has a positive effect on oxygen tension and EPO production [90]. Overall, OS driven by SMA revolves around hypoxia which emanates from reduced oxygen delivery due to reduced RBC's mass exacerbated by dysfunctional EPO synthesis.

Correction of both OS and SMA, as does AA, is pivotal to malarial disease amelioration and resolution through modulation of the immune system responses.

4.2 Immune system as mediator of SMA generation

While the immune system plays a fundamental part in erythropoiesis, in SMA, the immune response is central to its pathogenesis with pRBC's, hemozoin, and glycosylphosphatidylinositol (GPI) which activates monocyte and lymphocyte singly or jointly followed by increased inflammatory mediator synthesis. Pro-inflammatory mediators TNF-α, TNF-γ, IL-1 and IL-23 tend to be up-regulated while anti-inflammatory cytokines IL-4 and IL-10 exhibit low concentrations in SMA [91, 92]. The over expression of Th1 cytokines in malaria and SMA invariably affects erythropoiesis negatively and enhance OS. There is a close association between macrophage inhibiting factor (MIF) and SMA with bone marrow (BM) activity suppression influenced by NO as a powerful erythropoiesis inhibitor [93, 94]. Haemolysis and associated *Plasmodium* products like hemozoin intrinsically motivates SMA, erythropoiesis dysfunction, vasoconstriction which perturbs inducible nitric oxide synthase (iNOS) and increased EPO compensatory increases. However, increased EPO concentrations convoyed by insufficient erythroid progenitors response results in low reticulocytotic presentations [92, 95]. Ultimately, ineffective erythropoiesis, erythrophagocytosis and iron delocalisation may have a bearing on SMA [96]. Taken together, inflammatory responses and OS, which are corrected by AA administration, have a strong bearing in SMA outcomes.

Transdermal delivery of AA, as a once off application of a pectin hydrogel patch, influences hematocrit (Hct), a surrogate marker for SMA, suggests the phytochemical has influence on some yet unidentified mechanism of red blood cell (RBC) molecular level metabolism [79]. By influencing the causes of low Hct and SMA, which could be increased parasitaemia induced-haemolysis or inflammation induced-erythropoiesis-suppression, AA is able to address reduced RBC's concentration of malaria. Inflammation drives OS (and vice versa) and its attending outcomes through well-established mechanisms involving cytokines and other soluble effector molecules in a vicious cycle.

The TNF-α and other cytokines are well established inflammatory mediators. Reports of AA inhibition of TNF-α in acute pancreatitis in corneal lipoploysaccharide induced inflammation have been made. The phytochemical's antinociceptive activities and anti-inflammation in mice has been linked to its ability to inhibit cytokine activity [97–99]. Ultrasensitive fourth generation CRP concentrations have been shown to be significantly lower in *P. berghei* (murine malaria)-infected rats administered with both oral and transdermal amidated hydrogel matrix pectin patch as compared to untreated animals. This indicates an anti-inflammatory effect of the phytochemical [79] which is an essential anti-disease effect in malaria through inhibition of inflammatory markers like TNF-α.

Nonetheless, it has been shown that TNF-α causes hypoferraemia and reduces intestinal absorption of iron [100, 101]. A strong influence of SMA generation has been shown by increased concentrations of the cytokine in malaria [102] with a distinct mechanism for inadequate erythropoiesis linked to the cytokine [103]. Inflammation is a crucial driver of malaria where TNF-α is a key component, and SMA is a common complication [104] through erythropoiesis perturbations [105]. Therefore, it stands to reason that AA's influence on both inflammation and SMA could be through inhibition of TNF-α as an association between suppression of the cytokine by the phytochemical has been observed.

As a result, the relationship between AA and TNF-α in malaria present key proponents to malaria disease management. Notwithstanding, a similarly

structured triterpene, oleanolic acid (OA), has been reported to attenuate pro-inflammatory cytokines (TNF-α and IL-6) release and ameliorate anemia in murine malaria [106], properties that AA has been reported to have in other inflammatory conditions [107].

Ferroportin (FPN), an abundant protein in the reticuloendothelial system which mediates iron release and intestinal iron absorption, has its cytoplasm re-localisation induced by TNF-α [108]. This effect is obtained through working in tandem with hepcidin which is abundantly expressed in malaria and other chronic diseases with concomitant EPO resistance and dyserythropoiesis [109, 110]. Thus, possible inhibition of TNF-α and the known amelioration of anemia in malaria by AA may positively influence TNF-α-induced hypoferraemia which is driven by inflammatory mediators on hepcidin [101].

4.3 Ferroportin (FPN) and SMA

Interestingly, FPN expression on RBC's (54,000 copies per cell) has been reported as a novel finding for a protein that had not been thought to be found on these cells as export of Fe^{2+} had been thought to occur only after haemolysis of pRBC's [111]. By the exportation of iron generated by hemoglobin autoxidation, FPN protects against iron accumulation, as well as malaria infection and haemolysis creating an antioxidation status in the cell. In situations where there is increased inflammation, increases hepcidin synthesis which dysregulates FPN metabolism and increases Fe^{2+} accumulation within both the pRBC's and npRBC's promotes iron deficiency and haemolysis from OS [112]. The ability of AA to ameliorate malaria-associated inflammation and OS is critical in to the phytochemical's salvaging of Hct and SMA through preservation and or upregulation of FPN activity in both pRBC's and npRBC's. By reducing cellular Fe^{2+} in pRBC's parasites proliferation intracellularly is inhibited and protection against the disease conferred. Iron supplementation in malaria down-regulates FPN influencing growth of the parasite worsening malaria [113, 114]. Upregulation of FPN by AA is a possible mechanism by which the phytotherapeutic effects antiparasitic activity and anti-disease in murine malaria.

4.4 The inflammasome in chronic disease and asiatic acid administration

The inflammasome, a key component in the development of chronic disease anemia is characterized by anemia and decrease in RBC's volume corresponding with the patent period of murine malaria infection. During the patent period, rapid parasite multiplication is experienced culminating in peak parasitaemia and death. However, timely intervention with AA administration as a chemoprophylactic has salvages Sprague-Dawley (SD) rats from the brink of death to full recovery with 0% parasitaemia at Day 12–15 post infection [26].

Peak parasitaemia effects on infection outcomes are compounded by suboptimal EPO directed responses such as reduced cellular proliferation (with adequate EPO production) to SMA. The inflammasome seem to be the major underlying factor driving tissue insensitivity to EPO which leads to SMA even in acute cases. Administration of AA to reverses this process by restoring normal hemoglobin (Hb) concentrations and Hct.

4.5 Inflammasome drives SMA through hepcidin-ferroportin influence

Iron metabolism is influenced by inflammation of chronic disease through hepcidin, the inhibitor of the only known iron exporter. Inflammation drives the

synthesis of hepcidin in the liver with cytokines TNF-α, IL-1 and IL-6 playing a major role. Interestingly, the hepcidin phenomenon, by driving reduction in iron concentrations, is calculated to protect against severe *P. falciparum* malaria and death in young children [113]. Iron deficiency has also been shown to have a 5.5-fold protection against placental malaria fatalities [115]. Modulation of Fe^{2+} concentration resulting in iron deficiency uses hepcidin synthesis in chronic inflammation of malaria. This, therefore, means that inhibition of inflammation mediators will also influence hepcidin synthesis and thus remove the metabolic barricade on iron export by FPN.

The pleiotropic nature of AA comes to the fore in its ability to attenuate inflammatory cytokines (TNF-α. IL-1, IL-6) and combat malarial parasitaemia as well as influence iron metabolism through hepcidin. A direct causal relationship between hepcidin is and AA is yet to be established. However, it stands to reason that if AA is able to attenuate the drivers of hepcidin synthesis, it is also able to influence the hormone's metabolism. Subsequently AA may be able to influence iron metabolism through the same process of FPN-hepcidin interaction and reduction of iron export from enterocytes, macrophages, hepatocytes and RBC's. Preservation and recovery of SMA evidenced by normalization of Hct and other RBC indices (reported elsewhere) in malaria and parasitaemia reduction by AA indicates the possibility of its influence on FPN effects on iron export lost in pRBC's and was recovered for normal intracellular iron to take place.

There is an abundance of FPN on RBCs with effects on RBC iron status, and when down-regulated by hepcidin [111, 112] or other inhibitor could therefore influence the growth of malaria parasites [116, 117]. However, FPN down-regulation in malaria mediates parasitaemia proliferation, increase intracellular OS from accumulation of haeme Fe^{2+} [111]. The mutation of FPN Q24H (glutamine to histidine switch at position 248) is prevalent in sub-Saharan Africa populations with a prevalence of between 2.2–20% [118, 119] renders FPN resistant to hepcidin-induced degradation [120]. Due to the nonregulated form of the FPN, carriers tend to have lower hemoglobin concentrations than normal controls which is consistent with findings that high FPN levels in erythroblasts tend to export more iron, diminish hemoglobin synthesis [121, 122] although it may have protective effect against OS as a health benefit to those of African descent. Phytochemical administration in malaria and their effects on hepcidin-ferroportin relationship may have the same effect.

4.6 Mechanisms of oxidative stress, haemolysis and SMA in malaria

The underlying cause of malarial anemia does not get fully explained by dyserythropoiesis as it may have a rapid onset and life-threatening outcomes. A plausible explanation of reduced RBC mass includes the haemolysis associated with both pRBC's and npRBC's that occurs in malaria through changes that take place in the cell membranes.

While in the circulation, RBC's are in constant exposure to endogenous and exogenous reactive OS (ROS) with a high potential for cell damage and functional impairment through OS. However, ROS effects are minimized by the extensive antioxidant system involving non-enzymatic antioxidants of low molecular weight (glutathione and ascorbic acids as examples) and enzymatic antioxidants like superoxide dismutase, catalase, [123] glutathione reductase [124], and peroxiredoxin-2 [125, 126]. These antioxidants preserve the life span of RBC's through preserving cell membrane integrity [127]. The antioxidant ability to neutralize endogenous ROS is diminished when the blood flows through the microcirculation when hemoglobin (Hb) becomes partially oxygenated [128], more so in

malaria-infection cells. Also, partial oxygenation consequence in conformational changes of Hb and a dramatic increase in autoxidation of Hb building up with attending OS [129, 130]. Un-neutralized ROS in the RBC causes membrane damage that impairs their flow through the microcirculation and oxygen delivery to the tissues inducing hypoxia and OS generation [131]. Also, cells that come in contact with RBC's containing increased ROS tend to receive OS with subsequent tissue damage and inflammation induction [132, 133]. Indications are that RBC's NADH oxidase generate ROS from their membrane locations [134] away from the reach of cytoplasmic antioxidant enzymes.

One other none Hb autoxidation related OS leading to RBC deformity is associated with caspase 3. In the RBC, caspase 3 is activated by oxidative reactions with resultant degradation of band 3 [135, 136] which induce the exposure of phosphatidylserine, usually located in the inner leaflet of RBC membrane, to the outer surface [137]. The resultant histrionic reorganization of the membrane is concomitant with decrease in the discoid cell deformability [138] and ultimate haemolysis and RBC's mass reduction.

4.6.1 Oxidative stress, ATPases interaction and SMA

Ionic movement and homeostasis play a critical role in the development of OS and ensuing sequalae driving SMA. Inhibition of Ca^{2+}ATPase by OS [139, 140] enhances intracellular calcium concentration and activity with resultant RBC deformability decreases. Deregulated intracellular calcium concentration tend to activate the Gardos channel that results in potassium leakage from the RBC with subsequent cation homeostasis destabilization [128, 141] and shrinkage of cell with impaired deformability [128].

In malaria the total antioxidant capacity is compromised in the later stages of erythrocytic parasite development with membrane damage and breeches occurring through increased OS in both in npRBC's and pRBC's leading to SMA [142] as the RBC seem to be the readily available ROS sink in the disease [2]. This contributes to premature RBC's senescence and poor deformability resulting in the cells' splenic entrapment in the complex macrophage-abundant red pulp fenestrations [142, 143]. Oxidative stress is also associated with increased cell volume and density [144]. The growing parasite also increases OS and cell density that causes RBC microvesiculation as deformability and flexibility decreases exponentially. Any alterations that affects RBC volume and excess surface area tend to affect deformability of the cell [128] and this is a common occurrence in malaria leading to haemolysis, RBC concentration and SMA.

By interfering with parasite proliferation in murine malaria, AA directly contributes to normalizing cell volume and surface areas which aspects preserves cellular morphology in malaria [79, 145].

The induction of inducible nitric oxide synthase (iNOS), which increases NO synthesis, is closely linked to the inflammatory response in malaria with vascular permeability, pulmonary oedema and SMA as outcomes [146]. Increasing NO synthesis causes inhibition of the Na^+/K^+ ATPase with subsequent disturbance of water homeostasis in both the npRBC's, pRBC's and other tissues [147, 148]. When ATPase pump fails there is an accumulation of Na^+ in the intracellular compartment of both npRBC's and pRBC's with ensuing increased cell volume and surface area, membrane rigidity and reduced deformability and infiltrability in the spleen. Concomitant to decreased RBC's filterability is their removal by the spleen leading to SMA [27, 149]. ROS and $ONOO^-$ from inflammatory processes of malaria may have the same effect through oxidation of cell membranes and inhibition of the Na^+/K^+ ATPase pump with consequential SMA [150, 151]. Notwithstanding ATPase

inhibition, universal ATP depletion in malaria also uncouples the enzymes followed by RBC's membrane deformities, haemolysis and acute SMA [96, 152].

The fundamental basis of SMA driven by npRBC's and pRBC's spleen sequestration is underpinned by a synergistic effect of inflammation, OS (ROS and NS) and ATP depletion, falls under the ambit of disease processes resolved by the pleiotropic AA through its anti-inflammatory, antioxidant and neuroprotective roles [97, 107, 153]. There is an intriguing coordination of immune and erythropoietic responses in malaria related OS played by the AA administration which speculates extra dimensions in controlling parasitaemia and alleviation of SMA.

4.7 Malarial hypoglycaemia expression and the oxidative stress phenotype

Childhood *P. falciparum* malaria syndrome is preceded by a strong expression of hypoglycaemia. The etiology of low glucose concentrations in malaria have been accredited to antimalarial drugs like quinine and chloroquine which exhibit insulinomimetic action although patients without either intervention or disease have shown incapacitating hypoglycaemia [154]. Low blood sugar in malaria has also been attributed to increased consumption by growing parasites, but hypoglycaemia has been reported in low parasite loads in humans and it has also been shown that parasite only consume about 10% of the total plasma glucose even in severe malaria [155, 156].

A good and well characterized correlation exists in malaria between hyperlactaemia, hypoglycaemia and parasitaemia [157]. The possibility of microvasculature obstruction contributing to tissue hypoxia with attendant inefficient glucose utilization is plausible. Nevertheless, annotations that normal overall blood flow in the brain during periods of coma in malaria have been indicated [158]. Low blood flow rates in areas adjacent to high blood flow areas may elucidate the anomaly, however, hyperlactaemia requires a different explanation than the microcirculation obstruction alone and most likely a synergy with cytokine-induced oxygen underutilization could be involved [159, 160]. Elevated TNF-α has been associated with hypoglycaemia, hyperlactaemia and non-respiratory acidosis (nRA) in a number of diseases not related to malaria microvasculature obstruction as well [161]. Deliberate intervention with TNF-α in animals models tend to induce the same parameters [162, 163].

A causal relationship of hypoglycaemia and TNF-α may be intimated in malaria [164]. *Borrelia recurrentis* infection tends to elevate TNF-α in association with inflammation and the triumvirate of hypoglycaemia, non-respiratory acidosis and hyperlactaemia although there will not be parasites to excrete lactate or cause microvasculature occlusion [165].

Overall, TNF-α seem to be the main orchestrator of inflammation and that the anti-inflammatory effects of AA through inhibition of inflammatory mediators influence hypoglycaemia amelioration in malaria [30]. Indeed, the biologically active pentacyclictriterpenoid compounds oleanolic acid (OA) and maslinic acid (MA) have been shown to clear parasitic infection and ameliorate hypoglycaemia associated with malaria [106, 166] (**Figure 5**).

Asiatic acid and MA influence on glucose homeostasis in murine malaria involves the attenuation of glycolytic hormone activity by, in part, inhibiting glycogen phosphorylase (GP). Asiatic acid binds at the allosteric activator site naturally occupied the physiological activator AMP [30, 167]. This way glycolytic oxidation of glucose is reduced through reduced substrate while glycogen synthesis is upregulated. Mitochondrial associated OS may be reduced by inhibiting GP, as happens with glycogen synthase kinase 3β inhibition in chronic myocardial ischaemia or hypoxia [168], under hypoglycaemic conditions of malaria that would have rather

increase glucose mobilization being the norm. Actually, glycogen stores tend to be high in animals that are administered with AA as compared to non-treated controls, in experiments mentioned above showing glycogen preservation [30]. Inactivation of GP not only reduce glycogenolysis but also stimulates glycogen synthesis [167] preserving normoglycaemia in malaria and normoinsulinism. **Figure 6** indicates the allosteric binding site for the inhibitor AA on the dimeric GP.

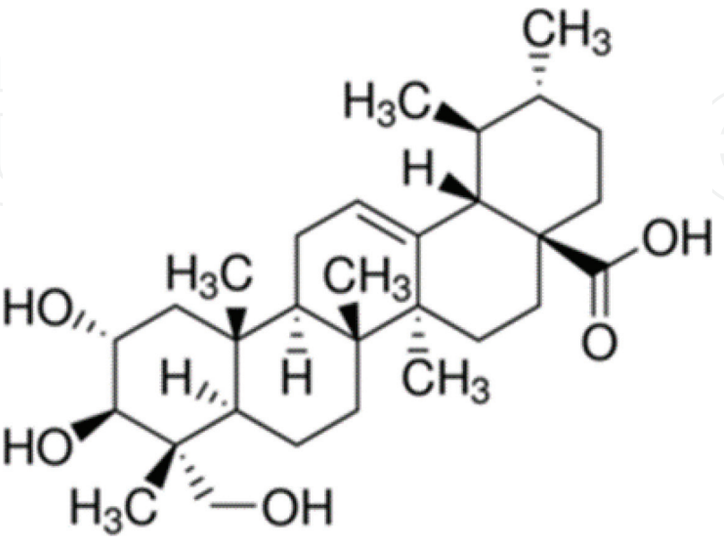

Figure 5.
Asiatic acid.

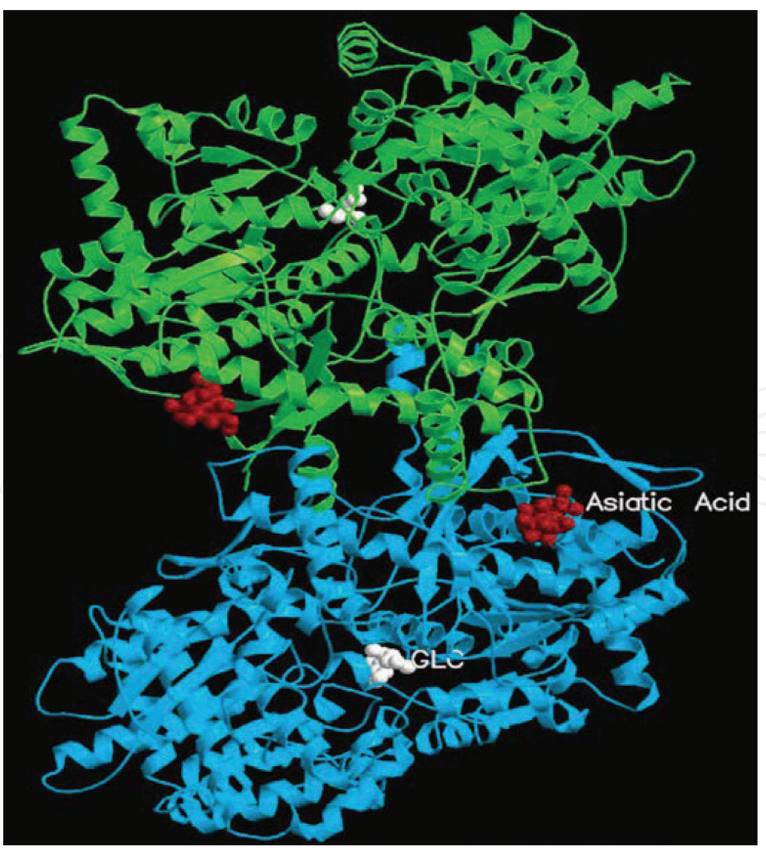

Figure 6.
Schematic diagram of the glycogen phosphorylase dimeric molecule, for residues 10–837, viewed down the molecular dyad. The catalytic (marked by GLUCOSE in white) and the allosteric (binds AMP and the inhibitors AA shown in red) binding sites positions are indicated. The allosteric site is situated at the subunit-subunit interface some 30 Å from the catalytic site [167].

The *Plasmodium* parasite, immunological and inflammatory responses, as well as chemotherapeutics currently used cause hypoglycaemia in malaria. The anti-hyperglycaemic, antioxidant, pro-oxidant properties useful in glucose homeostasis, observed when AA is administered in malaria, is hinged possibly on its influence on controlling hormonal as well as enzymatic aberrations seen in malaria and its treatment as explained above, where chloroquine and quinine treatment of malaria has been implicated with hyperinsulinism driving hypoglycaemia [169, 170].

A pre-clinical experiment showed that insulin resistance, which invariably causes hyperinsulinism [171], was ameliorated after administration of AA in malaria [30]. Oral administration of AA at 10 mg kg maintained normoinsulinism with reciprocal activity of glucagon in murine malaria in this study. Moreover, AA attenuated key glycolytic enzymes in streptozotocin-induced diabetes mellitus [172] an aspect that was seen in murine malaria with an overall effect on glucose tolerance response. Also, administration of AA terminated the satiation effects of high glucagon concentrations [173]. Also, positively modulated by AA administration, was the alleviation of the otherwise negative effects of malaria on food intake and weight gain.

Malaria induced-hypoxia producing hyperlactataemia was also ablated in animals that were administered with AA giving an overall high-grade wellbeing as opposed to severe malaria infection [174].

5. Malarial acute kidney injury (AKI) and oxidative stress

Kidney injury, acute or chronic, is one of the common differential diagnosis of malaria manifesting as syndrome. The diverse presentations and aetiological mechanisms of AKI orbit around the properties of pRBC's on microcirculation, hypovolaemia, metabolic derangements or host immunologic responses to infection [175–177]. These major pathogenic features are originated by the *Plasmodium* infection but may not be limited by the annihilation of the infection. Malaria associated AKI may develop after parasite abolition [178] necessitating interventions that also eradicate disease effects after parasitaemia clearance. Oxidative stress plays a critical role in AKI etiology driven by either the parasite or the immunological response to malaria infection which require neutralization in malaria. The source of kidney impairment in malaria is through direct tissue injury or inhibition of key components of kidney function. Inflammation as an immunological response initiator or vice versa has a close link to AKI development through OS initiators free haem Fe^{2+} and hemozoin which generate OS. Disruption of Na^{+} transporters by OS in the kidney [179] results in sodium wasting syndrome (hypernaturia), hypovolaemia with severe dehydration and non-respiratory acidosis leading to hyponatraemia and reduced glomerular filtration in malaria infection [180, 181] and malaria treatment with chloroquine complicates the disease [182, 183]. Therefore, renoprotective agents in the management of malaria AKI are imperative.

By combining the amphiphilic AA and amidated pectin hydrogel matrix in a transdermal drug delivery system provides a robust framework for combating malaria with renal function and electrolyte preservations [80]. AA-hydrogel matrix administration confers "an apply and walk away" once-off treatment for malaria as compared to the convoluted regimens of current antimalarial drugs in both dose, dosage frequency and administration route, not to mention the oxidative damage they bring. The non-nephrotoxicity of AA has been demonstrated in virtual screening experiments searching for selective inhibitors of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD 1) against 11β-hydroxysteroid dehydrogenase 2 (11β-HSD 2) [184].

These enzymes catalyze the interconversion of cortisone and cortisol in humans [185]. The isoform 11β-HSD 1 is located in the liver, adipose and brain where it converts the inactive cortisone to the active cortisol and 11β-HSD 2 is primarily expressed in kidney catalyzing the reverse conversion. The two enzymes provide a balance in glucocorticoid metabolism. AA was shown to selectively inhibit 11β-HSD 1 and not the other isoform. When 11β-HSD 1 is inhibited there is a resultant reduction in liver gluconeogenesis, lipophilia and there is improved insulin sensitivity [186], which may explain the positive influence of AA administration on glucose homeostasis in malaria [30]. Therefore, 11β-HSD inhibition by AA works in tandem with AA glycogen phosphorylase (GP) inhibition (see above) that preserve glycogen stores. Indeed, the kidney glycogen stores tend to be significantly higher in AA administered SD rats than untreated controls. Nevertheless, inhibition of 11β-HSD 2 leads to sodium retention, hypokalemia and hypertension [187] parameters which were not observed with AA administration as compared to controls in the transdermal drug delivery of AA studies above.

Further, selective inhibition of 11β-HSD 1 has been suggested to induce anti-inflammatory effect via the stimulation of haeme oxygenase-1 in LPS-activated mice and J774.1 in murine macrophages [188], which may explain the preservation of renal electrolyte handling that was observed when AA was administered. Electrolyte loss in malaria results from inhibition of Na^+/K^+ ATPase, ENac and other electrolyte channels by OS (ROS) in the proximal convoluted tubules which results in an increased sodium load reaching the distal convoluted tubes. Excess Na^+ is as a result lost in the urine in a what is referred to as pseudohypoaldosteronism. Therefore, a dual role of AA may be observed in malarial AKI in that the anti-inflammatory and antioxidant effect through the selective inhibition of 11β-HSD 1 with ultimate reduction in gluconeogenesis which reduce glycolysis and in lipid synthesis which reduces lipid peroxidation. Antioxidant capacity is seen through AA inhibition of glycogen phosphorylase and glycolysis modulation. Increased insulin insensitivity, that is usually seen in end-stage malaria fronted by increasing glucose concentrations and OS, is abolished by AA inhibition of 11β-HSD 1. Furthermore, glycogen synthase upregulation when glycogen phosphorylase is inhibited increases glycogen storage in the kidneys and this way restoring optimum renal function and electrolyte handling deranged by OS driven AKI. Also, inhibition of 11β-HSD1 promotes autophagy and correlates with reduced OS, inflammation [189] which are key pundits in malarial AKI eradicable by AA administration.

6. Conclusion

Oxidative stress drives malaria pathophysiology by ROS and NS insults upon pRBC's and npRBC's from parasitic infection, immunological host response. The inflammatory milieu has a cross cutting foot print in malarial syndromes intricately intertwining complex disease events, processes and systems to bring about malaria disease. Here we have shown how artemisinin, a commonly used antimalarial phytotherapeutic and asiatic acid, an experimental antimalarial phytochemical, to explain their various interactions in the combat of malarial disease.

Asiatic acid, armed with constitutive antioxidant and oxidative properties inhibit parasitic growth, host inflammasome and ameliorates systemic abnormalities in malaria. With selective enzymatic inhibition propensities, apoptotic influences and amelioration of malaria-induced systemic metabolic derangements, AA shows potential as an anti-parasitic, anti-disease, anti-inflammatory, antioxidant, immunomodulatory, renoprotective and malarial disease elixir.

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Abbreviations

AA	asiatic acid
ARF	acute renal failure
SMA	severe malaria anemia
nRA	non-respiratory acidosis
NO	nitric oxide
iNOS	inducible nitric oxide synthase
$ONOO^-$	peroxynitrite
ROS	reactive oxygen species
pRBC's	parasitized red blood cells
npRBC's	non-parasitized red blood cells

Author details

Greanious Alfred Mavondo[1*], Blessing Nkazimulo Mkhwanazi[2], Joy Mavondo[3], Wisdom Peresuh[4] and Obadiah Moyo[5]

1 National University of Science and Technology, Faculty of Medicine, Pathology Department, Bulawayo, Zimbabwe

2 University of KwaZulu Natal (UKZN), Pietermaritzburg, South Africa

3 Imagegate Diagnostics (PL), Bulawayo, Zimbabwe

4 Labnet Laboratories (P/L), Bulawayo, Zimbabwe

5 Chitungwiza General Hospital, Chitungwiza, Zimbabwe

*Address all correspondence to: greanious.mavondo@nust.ac.zw

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