

# Antimalarial Phytochemicals: Delineation of the Triterpene Asiatic Acid Malarial Anti-Disease and Pathophysiological Remedial Activities - Part II

Mavondo GA<sup>1,2\*</sup> and Kasvosve I<sup>3</sup>

<sup>1</sup>Pathology Department, Faculty of Medicine, National University of Science and Technology, Mpilo Hospital NUST Complex, Vera Road, Mzilikazi, Ascot, Bulawayo, Zimbabwe

<sup>2</sup>Discipline of Humana Physiology; School of Laboratory Medicine and Medical Sciences; University of KwaZulu Natal; Durban; South Africa

<sup>3</sup>Professor of Medical Science & Head of School; School of Allied Health Professions, Faculty of Health Sciences, University of Botswana, Gaborone, Botswana

**\*Corresponding author:** Mavondo GA, National University of Science and Technology, Faculty of Medicine, Division of Basic Medical Sciences, Mpilo Hospital Complex, Vera Road, Mzilikazi, Bulawayo, Zimbabwe, Cell +263775540788; +263716151185; E-mail: greaniousa@gmail.com; greanious.mavondo@nust.ac.zw

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## Abstract

Malaria is a composite condition of the *Plasmodium* parasite infection and accompanying pathologies. Parasite induced red blood cell perturbations and immunological response to infection drive various organ-specific syndromes accounting for a huge percentage of deaths amongst children <5 years and pregnant women. The multi-factorial pathophysiology includes acute renal failure, hypoglycaemia, severe malaria anaemia, acute respiratory distress syndrome/ acute lung injury and cerebral malaria as some of the prominent presentations of the disease. Current malaria treatment has largely remained “anti-parasite” or “anti-infection” necessitating discovery of “anti-disease” drugs that will ameliorate immunological aberrations, inflammation and metabolic disturbances which are ultimately the cause of high morbidity and mortality. Asiatic acid, a phytochemical, has well known curative properties on other conditions which share disease manifestations with malaria. However, the influence of Asiatic acid on malaria has not yet been reported. This review unravels the different facets of Asiatic acid and their possible remedial effects on molecular and biological changes introduced by the disease with emphasis on how this relates to glucose metabolism, acute renal failure, severe malaria anaemia, acute respiratory distress syndrome/ acute lung injury and cerebral malaria.

**Note:** Antimalarial phytochemicals: delineation of the triterpene Asiatic acid malarial anti-disease and pathophysiological remedial activities - Part II should be ready in conjunction with Antimalarial phytochemicals: delineation of the triterpene Asiatic acid malarial anti-disease and pathophysiological remedial activities - Part I

**Keywords:** Asiatic acid; Phytochemical; Malaria; Anti-Disease; Antioxidant; Ant-Parasitic; Anti-Disease

**List of abbreviations:** AA: Asiatic acid; ARF: Acute Renal Failure; SMA: Severe Malaria Anaemia; ARDS/ALI: Acute Respiratory Distress Syndrome/Acute Lung Injury; CM: Cerebral Malaria; Nra: Non Respiratory Acidosis; NO: Nitric Oxide; iNOS: Inducible Nitric Oxide Synthase, ONOO-: Peroxynitrite; ROS: Reactive Oxygen Species; OS: Oxidative Stress

## Introduction

The malaria parasitological mediator is the *Plasmodium* parasite which enterprises a haematological disease of monstrous complexity trailing a mortality rate second only, if not higher, than that of the HIV/AIDS pandemic especially in under developed countries [1,2]. Morphological and functional changes of the erythrocyte, immunological response to infection and the inflammatory mediation coordinates organ-specific syndromes, that are experienced in malaria account for the high morbidity and mortality among children <5 years and pregnant women in malaria-endemic areas [3-6]. The clinical sequelae include acute kidney injury (AKI), hypoglycaemia, severe malaria anaemia (SMA), acute respiratory distress syndrome/ acute lung injury (ARDS/ALI) and cerebral malaria (CM) as some of the prominent presentations of the disease [7-16]. Most of these disease manifestations are also as a result of treatment that tends to impinge on natural body defence systems and function in malaria worsening disease prognosis.

Current malaria treatment is mainly “anti-parasite” or “anti-infection” when paralleled to anti-disease prospects of treatment. This makes the discovery of new “anti-disease” drugs that will ameliorate the pathophysiological manifestations which are ultimately the cause of high incapacitations and death rates. Asiatic acid (AA), a phytochemical, has well known curative properties on other conditions which share disease manifestations with malaria [17-20]. Reports on the influence of AA on malaria are slowly emerging. Administration of the phytochemical has been mainly oral or as topical ointment for wound healing. Systemic circulation administration through transdermal delivery systems (TDDS) has been added to its list of administration options. The current review, Part II of a two part work, reconnoitres the potential therapeutic effects of AA biological changes introduced by the malaria disease, highlighting how this may relate to hypoglycaemia, acute kidney injury (AKI), severe malaria anaemia (SMA), acute respiratory distress syndrome/ acute lung injury (ARDS/ALI) and cerebral malaria (CM).

Triterpenes are synthesized through the combination of isoprenoids (C<sub>5</sub>H<sub>8</sub>) [21]. These are a group of phytochemicals which characteristically exhibit pentacyclic phenolic compounds with substitutable functional groups enabling them possible interaction with a variety of substances. Natural triterpenes are secondary metabolites of plant species with selective oxidant and antioxidant properties [22]. Triterpenes have been suggested to possess oxidative properties, in the same way with artemisinin and analogous antimalarial pharmaceuticals [23]. This way, triterpenes may mimic the evolutionary effects of haemoglobinopathies (glucose-6-phosphate dehydrogenase deficiency, sickle cell disease) on the infected red blood cell (pRBC's) environment that kill the parasite by altering its oxidative status [24,25].

## Systemic Disease and AA administration

### Severe malaria anaemia (SMA) and Anti-disease effects of AA activity

SMA, a major cause of morbidity and mortality, has a multifaceted aetiology. There are two potential mechanisms contributing to malarial anaemia: increased destruction of pRBC's and nRBC's (immune mediated haemolysis, phagocytosis, splenic sequestration) and decreased RBC's synthesis from both immune system and parasite effects [26-28]. Under normal physiology, the number of red blood cells destruction is counter balanced with red blood cell production denoted by an efflux of reticulocytes from haematopoietic tissues. Erythropoietin (EPO) production in kidneys is normally up-regulated when anaemia develops from increased RBC's destruction such as haemolysis or haemorrhage with a concomitant increase in reticulocytosis and rheological disturbance alleviations. Besides EPO, other growth factors and cytokines, including granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF), insulin-like growth factor-1 (IGF-1) and cytokines are involved in erythropoiesis [29-31].

Normal kidney physiology is necessary for the production of EPO in the peritubular fibroblasts of the renal cortex [32,33]. Production of EPO is regulated by tissue oxygen tension in a feed-back loop with haematocrit, centred on an inverse logarithmic relationship [34]. Correction of haematocrit by AA may have a positive effect on oxygen tension and EPO production.

While the immune system plays a pivotal role in erythropoiesis, in malarial anaemia, immune response is central to its pathogenesis with pRBC's, hemozoin, and GPI activating monocyte and lymphocyte. Pro-inflammatory mediators TNF- $\alpha$ , TNF- $\gamma$ , IL-1 and IL-23 are up-regulated in malarial anaemia. Anti-inflammatory cytokines IL-4 and IL-10 display low levels in severe malaria [26,35]. Macrophage inhibiting factor (MIF) is associated with severe anaemia with bone marrow (BM) suppression and NO being a potent erythropoiesis inhibitor [36,37]. High levels of EPO accompanied by inadequate erythroid progenitors response results in low reticulocytosis [35,38]. Ineffective erythropoiesis, erythrophagocytosis and iron delocalisation may have the same effect [10]. Taken together, inflammatory responses, which may be ameliorated by AA administration, have a strong bearing in SMA.

Transdermal delivery of asiatic acid as a pectin hydrogel patch has been reported influence haematocrit (Hct), a surrogate marker for SMA which could suggest that AA had influence for some aspect of red blood cell metabolism [39]. The influence of AA on Hct and SMA may be due to its effect on the causes of SMA which could be increased parasitaemia induced-haemolysis or inflammation induced-erythropoiesis-suppression. AA has been shown to influence inflammation in malaria which could mean amelioration of SMA. There is scarcity of data that links asiatic acid and erythropoietin directly showing gaps in information that need further research. This review seeks, among other issues, to open up more research on malaria and asiatic acid with the hope of unveiling more information on the triterpene's influence of the disease. The mechanism by which AA influences SMA still needs to be unraveled. The influence of AA in TNF- $\alpha$  and other inflammatory mediators is without doubt. AA has been reported to influence inflammation through inhibition of TNF- $\alpha$  in acute pancreatitis in corneal liposaccharide induced inflammation, has antinociceptive activities and its mechanisms of anti-inflammation mice through the same molecule [40-42]. In malaria AA has been shown to influence inflammation as demonstrated by lower CRP concentrations when AA is administrated as compared to when it is absent [39]. Therefore, if it has been shown that TNF- $\alpha$  causes hypoferraemia and reduces intestinal absorption of iron, it may follow that this may influence anaemia development. Malaria is driven by inflammation, where TNF- $\alpha$  is a key component, and SMA is a common complication. AA's influence on both inflammation and SMA could have been through inhibition of TNF- $\alpha$  although such a causal relationship has not been demonstrated in malaria to date. As a result, the relationship between AA and TNF- $\alpha$  in malaria is an area warranting future exploration. However, oleanolic acid (OA), a triterpene like AA, has been reported to attenuate pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) release and amelioration of anaemia in murine malaria properties that AA has been reported to have in other inflammatory conditions.

Cytoplasm re-localisation of ferroportin (FPN), an abundant protein in the reticulo-endothelial system which mediates iron release and intestinal iron absorption, is induced by TNF- $\alpha$  possibly working in tandem with hepcidin which is abundantly expressed in malaria and other chronic diseases with concomitant EPO resistance and dyserythropoiesis. Hypoferronaemia from reduced iron macrophages release and absorption from the intestines also contribute to malarial anaemia through hepcidin binding of FPN and reducing iron export from macrophages [43-45]. Inhibition of TNF- $\alpha$  in malaria by AA may have a positive influence on TNF- $\alpha$ -induced hypoferraemia [46].

There seems to be a paucity of information on the effect of AA in the metabolism of iron in malarial anaemia, which facet may need to be investigated seeing that the phytomedicinal agent influences the inflammasome, a key component in the development of chronic disease anaemia. The setting in of anaemia and decrease in RBC's volume corresponds with the patent period of murine malaria infection where there is rapid multiplication of the parasite culminating in peak parasitaemia, a process that may be inhibited by timely treatment with AA or its use as chemoprophylactic [47]. Compounding this information, insufficient erythropoiesis in malarial anaemia driven by inflammatory mediators with adequate EPO production but suboptimal response, seem to be the major underlying factors to SMA anaemia which may be reversed by AA administration. Iron metabolism is influenced by inflammation of chronic disease through hepcidin, the only known iron exporter, secretion which can be influenced by the anti-inflammatory effect of AA. Hepcidin secretion is influenced by inflammatory cytokines, (TNF- $\alpha$ , IL-1, IL-6) which have been shown to be attenuated by AA administration in malaria and other inflammatory conditions. Therefore, AA may affect iron metabolism in these inflammatory conditions although this needs to be established in future studies.

Dyserythropoiesis does not fully explain or account for the underlying cause of malarial anaemia which may have a rapid onset resulting in life threatening incidences. Acute loss of nprBC's, whose contribution to malarial anaemia has been calculated, is a plausible cause of SMA. Premature RBC's senescence and poor deformability, caused by a number of factors, result in their splenic entrapment in the intricate macrophage-abundant red pulp fenestrations [48-49].

The induction of iNOS, as an inflammatory response in malaria increases NO contributing to the development of malarial anaemia, vascular permeability and pulmonary oedema [50,51]. Increased levels of NO inhibits the Na<sup>+</sup>/K<sup>+</sup> ATPase necessary for the maintenance of water balance between the intracellular and extracellular compartments of the nprBC's, prBC's and other tissues [52,53]. ATPase pump failure results in accumulation of Na<sup>+</sup> in the intracellular compartment of both nprBC's and prBC's with ensuing membrane rigidity or reduced deformability, as water gathers in the cell. Paralleled by decreased RBC's filterability is their removal by the spleen leading to SMA [54,55]. ROS and ONOO<sup>-</sup> from inflammatory processes of malaria may have the same effect through oxidation of cell membranes and inhibition of the Na<sup>+</sup>/K<sup>+</sup> ATPase pump with consequential SMA [56,57]. Besides ATPase inhibition, general ATP rundown of malaria also decouples the enzymes with subsequent RBC's membrane deformities, haemolysis and acute SMA [10,58].

SMA resulting from nprBC's and prBC's spleen sequestration has underlying factors of inflammation, OS (ROS and NO) and ATP depletion working in synergy, indicates a disease process with a high probability of being resolved by AA through its anti-inflammatory, antioxidant and neuroprotective roles [59-61]. However, the mechanism of action of the proposed alleviation of malaria-related ills is unknown. The hall mark of current treatment regimens is parasite clearance but lacks disease-free status guarantee [47]. AA treatment posits a possibility of an intriguing coordination between the immune and erythropoiesis responses as extra dimensions in controlling parasitaemia and alleviation of SMA.

## Acute respiratory distress syndrome (ARDS), acute lung injury (ALI) and AA

Accompanying ALI and ADRS is deep breathing, respiratory distress, pulmonary oedema which may develop before or after specific treatment [62-63]. In the tropics, malaria is the most common risk factor for ADRS and ALI and the second most prevalent cause of the disease after sepsis [64,65]. Subclinical impairment of lung function displayed by small airways obstruction, alveolar ventilation reduction, impaired gaseous exchange with increased phagocytic activity are common in uncomplicated malaria [66]. Inflammation activation seem to be written all over ADRS/ALI with high levels of vascular endothelial growth factor (VEGF), which may sustain increased vascular permeability causing malaria respiratory failure, having been reported in the disease [67]. An increased expression of TNF- $\alpha$ , IL-10, IFN- $\gamma$ , CXCL10 and CXCL 11 as well as monocytes and neutrophil chemoattractant chemokines (CCL 2 and KC) were demonstrated in malaria infected rats lungs [68]. While parasite sequestration was observed in the lungs, it was lower than in the brain [69]. We speculate that resolution of ADRS/ALI by AA administration is possible as the drug possess both anti-disease (immunity modulation, anti-inflammatory and antioxidant) and have indicated its anti-parasitic properties [70]. Indeed, dexamethasone, a steroidal anti-inflammatory, was observed to inhibit macrophages and CD8 T cells lung infiltration in ADRS although it does not have anti-parasitic activity [71].

Oedema development indicates an increased lung water content which may be related to decreased endothelial sodium channel (ENaC) expression emanating from hypoxia resulting from malarial anaemia or TNF- $\alpha$  mediated downregulation [68]. The inhibition of the Na<sup>+</sup>/K<sup>+</sup> ATPase pump by NO may also contribute to the collection of water in lungs causing or exacerbating the oedema. Net energy depletion and hypoxia, stimulated by inflammatory mediators may also lead to intercostal muscle weakness, as part of general muscle weakness status of malaria disease. This may also cause reduced lung perfusion and lung injury from accumulated toxins. By nature of anti-oxidative stress and anti-inflammatory activity, AA may be able to alleviate ADRS/ALI by quenching ROS/NO and ONOO<sup>-</sup> generated in malaria [59,63,72-74].

## Hypoglycaemia, hyperlactaemia, non-respiratory acidosis (nRA) and AA malarial treatment:

Hypoglycaemia is a key component of childhood *P. falciparum* malaria syndrome. The anti-hyperglycaemic effect of AA has been reported in streptozotocin (STZ)-induced diabetic rats where it was shown to mediate the release of glucose from glycogen and utilization in glycolysis [75]. However, its activity in normoglycaemic and hypoglycaemic states in malaria or other diseases is not well known. The development of low glucose concentrations in malaria have been attributed to antimalarial drugs like quinine and chloroquine which display insulinomimetic activity although patients without either treatment have shown a debilitating hypoglycaemia [76]. Consumption of glucose by the parasite burden has also been accused of causing low blood sugar in malaria 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 [77,78]. The severity of the malarial disease is well correlated with hyperlactaemia, hypoglycaemia and parasitaemia [79-81]. It is plausible that microvasculature obstruction may contribute to tissue hypoxia with concomitant inefficient glucose utilization. However, observations that overall blood flow in the brain was within normal during periods of coma in malaria have been made [80]. Although low flow areas adjacent to high flow areas could explain that anomaly, the hyperlactaemia may not be fully explained by the microcirculation obstruction alone but most likely by a synergy with cytokine-induced oxygen underutilization [81-83]. Of note, hypoglycaemia, hyperlactaemia and nRA may also be seen in a number of diseases, not related to malaria microvasculature obstruction, accompanied by elevated TNF- $\alpha$  [84]. Injection of TNF- $\alpha$  into animals models tend to elevate the same parameters [85,86]. In human malaria, hypoglycaemia is intimately associated with TNF- $\alpha$ , suggesting a causal relationship [87]. Coxon, *et al.* (1994b) has indicated that when TNF- $\alpha$  is elevated, as in inflammatory disease caused by *Borrelia recurrentis*, the triumvirate of hypoglycaemia, nRA and hyperlactaemia tend to coexist without parasites to excrete lactate or cause microvasculature occlusion [88]. This indicates that the anti-inflammatory effects of AA through inhibition of inflammatory mediators may influence the glucose metabolism in malaria as has been shown elsewhere [89]. Indeed, clearance of the parasite and amelioration of hypoglycaemia associated with malaria as has been observed with MA and OA [90,91]. AA has been reported to influence glucose homeostasis in murine malaria where the attenuation of hormonal activity was observed [89]. The *Plasmodium* parasite, immunological and inflammatory responses, as well as chemotherapeutics currently used cause hypoglycaemia in malaria. AA has anti-hyperglycaemic, antioxidant, pro-oxidant properties useful in glucose homeostasis. Malaria as well as chloroquine and quinine treatment of malaria has been associated in hyperinsulin secretion conditions that worsen hypoglycaemia of malaria [92-95]. Malaria also induces insulin resistance in uncomplicated cases which was ameliorated in murine malaria by administration of AA in an pre-clinical experimental study [89,96]. The maintenance of normal insulin in malaria and reciprocal concentrations of glucagon when 10mg/kg body weight was administered orally showed the influence of the phytochemical AA in malaria [89]. AA has been shown to attenuate key glycolytic enzymes in diabetes mellitus an aspect that was seen in murine malaria with an overall effect on glucose tolerance [73,89]. Administration of AA was also shown to modulate glucagon effects on food intake and weight gain by terminating the satiation effect of the hormone at increased levels that are associated with malaria [97]. Hyperlactaemia, a product of malaria induced-hypoxia was also ablated in animals that were administered with AA giving an overall high grade wellbeing that was not seen in severe malaria infection [98].

## Hyperlactaemia, a non-respiratory acidosis (nRA) and AA administration

There is an erroneous assumption of linking hyperlactaemia to nRA although the two emanate from two distinct and different, but subsequent events which need to be elucidated in the light of malaria, inflammation and AA treatment [99]. The term lactic acidosis is used to imply that lactic acid is being produced when both nRA and hyperlactaemia are present which is biochemically false. The anion lactate is formed together with ATP in anaerobic glycolysis and no hydronium ions are formed in the process [100]. Hyperlactaemia cannot cause acidosis and hyperlactaemia can exist with or without acidosis [101]. Hydrogen ions ( $H^+$ ) are generated on the hydrolysis of ATP and are used up in the regeneration of more ATP from ADP in the mitochondria. Mitochondrial failure will cause regeneration of ATP to take place in the anaerobic glycolysis where there is no consumption of  $H^+$ . As the buffering capacity is exceeded, nRA will ensue [102]. Inflammatory mediators (iNOS, NO and ONOO $^-$ ) being the drivers of mitochondrial dysfunction that give rise to nRA increase as hypoperfusion seen in systemic inflammation escalates from possible tissue injury [103]. Due to increased glycolysis, much more glucose is required to achieve the same energy levels of aerobic respiration [104]. Consequently, more glucose is consumed; more lactate generated and less  $H^+$  utilised driving the hypoglycaemia, hyperlactaemia, concurrently. Termination of inflammation, in malaria, may halt the triumvirate onslaught [105]. Leucocytosis may contribute to hyperlactaemia due to increased production and decreased clearance in hepatic failure meaning that hepatoprotection of AA may alleviate the metabolic derangements [106,107]. T cells activation and cytokine release is similar in both fulminant and malaria [108-110]. Macrophages are also influenced by AA in their production inflammatory mediators during inhibition of NO and prostaglandin E ( $PGE_2$ ) [111].

Use of pH as a surrogate marker of blood lactate in sick infants is rather inaccurate and it is conversely true for using lactate as indicator for acidosis [112]. After hypoxic episode the brain uses lactate and not glucose as energy source for the recovering synaptic function and may be the host's natural mechanism to protect against hypoglycaemia [113-116]. Hyperlactaemia in malaria may illustrate the metabolic derangement in the patient than a risk factor for nRA [58]. Indeed, treatment of non-malarial multi-organ disease hyperlactaemia with dichloroacetate did reduce the levels of the anion but with little survival impact [28].

There is a possibility that AA may attenuate nRA, hypoglycaemia and hyperlactaemia by inhibiting inflammatory cascade as the phytopharmaceutical's anti-inflammatory and carbohydrate metabolic effects have been observed [60,74]. Taken together, the given view is that of inflammation in malaria being a potent driver of hypoglycaemia, hyperlactaemia and nRA which aspects may be attenuated by the immunomodulatory capacity of AA.

### Glucose transportation in malaria and AA influence

Glucose transportation and consumption are amplified by GPI, TNF- $\alpha$ , and IL-1 by increasing expression of GLUT-1 transporters on cell membranes and insulinomimetic activities which invariably influences hypoglycaemia in malaria [117-119]. Stimulation of fructose 2, 6-bisphosphate by inflammatory cytokines up-regulates phosphofruktokinase-1 (PFK-1), a glycolysis rate-limiting enzyme, increases the glucose anaerobic oxidation which, in the face of mitochondrial failure, may worsen non-respiratory acidosis (nRA), hypoglycaemia and hyperlactaemia [120]. Anaerobic glycolysis yields more lactate, worsen hypoglycaemia when hepatic glycogen is exhausted, and nRA becomes evident.

The effect of AA on these events in malaria is not yet elucidated. However, anti-hyperglycaemic activity, which may not necessarily mean hypoglycaemic action, has been reported in STZ-induced diabetic rats. The triterpene attenuated the activities of key carbohydrate metabolism enzymes in glycolysis, glycogen synthesis and gluconeogenesis [75]. Nevertheless, underlying mechanism of AA interactions with these enzymes requires elucidation. It is also unknown whether AA acts directly or stimulates insulin secretion or acts in synergy with the hormone as does glibenclamide through inhibition of ATP sensitive K<sup>+</sup> channels resulting in overall glucose homeostasis regulation in liver and skeletal muscles [121,122].

Malaria infection enhances insulin sensitivity through the upregulation of inflammatory cytokines, as previously mentioned, and how this plays out with AA's envisaged plasma glucose lowering effect requires determination. However, inhibition of inflammation driven cachexia may correct both hyperparasitaemia and glucose homeostasis. Ursolic acid, an AA family member but with no known antimalarial action, and 18 $\beta$ -glyrrhethinic acid have been shown to possess insulinomimetic activities in STZ-induced DM rats [121,123].

### Kidney disease in malaria and AA treatment

Renal failure is one of the common differential diagnosis of malaria manifesting as either a chronic or acute syndrome. The varied presentations and aetiological mechanisms revolve around the effects of pRBC's on microcirculation, hypovolaemia, metabolic derangements or host immunologic responses to infection [124-126]. These principal pathogenic features are initiated by the *Plasmodium* infection but may not be limited by the eradication of the infection. Malaria associated renal failure may develop after parasite clearance [124]. This understanding suggests the use of anti-disease treatment regimens, alone or in combination with anti-parasitic drugs, as an effective anti-malaria treatment approach. Immune modulatory and anti-inflammatory compounds, like AA and other triterpenes, may attenuate immune complex formation and their deposition in the sub-endothelium averting mesangio-capillary glomerulonephritis seen in chronic malarial nephropathy and tubular necrosis of ARF, sequelae of post-treatment immune reactivity [127-128].

### Cytoadherence down-regulation, acute renal failure (ARF) and AA

The var gene super family member parasite ligand PfEMP-1 expression on pRBC's selectively adhere with constitutively and induced cytoadhesins (CD36, ICAM-1, VCAM-1, CSA) in the renal endothelium intima anchoring the cells in the microvasculature [129-131]. Parasite infected RBC's find a ready attachment domain in CD36 which is abundantly expressed in the kidneys with consequent sequestration of both npRBC's and pRBC's in the post-capillary venules and capillaries of the renal system [132,133]. Resultant rheological perturbations from sequestration and microvasculature occlusion in the kidneys activate local release of cytokines, ROS, NO and ONOO<sup>-</sup> with induction of tubular lesions leading to ARF [12]. Sodium wasting and pseudohypoaldosteronism may develop leading to hyponatraemia and hypernatruria [90,134]. Cytoadherence is accelerated by febrile paroxysms of malaria through up-regulation of PfEMP-1 reflecting a pro-inflammatory (Th1) mediation, meaning that AA's anti-inflammatory and antioxidant properties may alleviate malaria AKI [135].

The production of adhesive molecules in malaria is mediated in part by the immune and inflammatory processes when vascular endothelial cells (ICAM-1 and VCAM-1) in most regional circulations assume an inflammatory phenotype. This process is mediated in a tissue-specific manner by cytokines and immune cells [136]. Therefore, as AA has immunomodulation and anti-inflammation capacity, it follows that the phytochemical may possess the potential to inhibit adhesive molecules-endothelial cells complex formation in malaria.

### Oxidative stress, acute kidney injury (AKI) or renal failure (ARF) and AA

It is generally accepted that cytoadhesion and clogging of the capillaries by pRBC's *per se* plays a marginal role in the pathogenesis of ARF as the extent of RBC's sequestration in the glomerular and tubulointerstitial capillaries has been observed to be far less than in CM [137]. OS has a high potential for renal cytotoxicity induction. Indeed, mononuclear cells have been reported to accumulate in the glomerular and peritubular capillaries, which when activated, release cytokines, ROS and NO stimulating local host immune

responses, leading ARF [138]. Furthermore, late stages of the severe malaria results in unbalanced host mechanical, immunological and humoral response with increased ROS and NO synthesis overrunning antioxidant defence systems [139-141]. The deleterious pathological consequences, by an otherwise protective process, result in ARF [142]. Hyponatraemia, hyperkalaemia, adrenal insufficiency in malaria coincides with high iNOS activity culminating in acquired pseudohypoaldosteronism type 1 prompted by generated NO and subsequently ONOO<sup>-</sup> which inhibits ENaC and Na<sup>+</sup>/K<sup>+</sup> ATPase pump in the PCT [52,53,143]. Ischaemic damage the radicals may have on the tubular interstitium and peritubular tissues ultimately leads to ARF when there is no proper intervention. Failure of the ATPase pump affects the Na<sup>+</sup>/K<sup>+</sup>/H<sup>+</sup> exchanger with resultant bicarbonate (HCO<sup>-</sup>) loss, Na<sup>+</sup> wasting and H<sup>+</sup> proton retention, nRA and ARF. AA may downregulate ROS, NO and ONOO<sup>-</sup> generation alleviating severe malaria, ARF and Na<sup>+</sup>/K<sup>+</sup> ATPase pump inhibition. AA has a potential of ameliorating OS and ARF as it has diuretic effects although mechanisms of action is still obscure.

## Severe malaria anaemia (SMA) and kidney failure

*Plasmodium* infection, being a blood borne disease, the rheological aspects of blood fluid determines abilities to response to gaseous exchange challenges, nutrients and waste homeostasis, disease and treatment outcomes. Prevention, treatment and resolution of factors contributing to anaemia becomes paramount to the resolution of disease aspects of malaria. SMA is seen early in the parasitic infection and predisposes to renal insufficiency. SMA-induced ARF emanates from hypovolaemia instituted by low blood cell counts and volume, decreasing blood flow and subsequently glomerular filtration rate (GRF). The compensatory blood volume replenishment includes increased synthesis of plasma proteins, like fibrinogen, which increases blood viscosity exacerbating sluggish blood flow and reduced oxygen supply to the interstitium, driving ischaemic activities and ARF [144]. Research has shown that AA administration modulated SMA and SMA development, but the mode of action of this phytochemical has not yet been established to date [39]. However, by modulating inflammatory mediators, which are the drivers of SMA through inhibition of iron absorption and delivery to the circulation this suggests that AA may influence SMA development through its anti-parasitic and anti-inflammatory effects. As stated elsewhere, AA has immunomodulation and apoptotic capacity which may influence leucocyte cellular concentration and relieve algid malaria development as has been observed with the murine experiment. AA has been reported to alleviate SMA in *P. berghei* infected Sprague-Dawley rats which may explain the phytomedicinal compound's possible influence on acute renal injury [145-147].

## RBC's destruction in SMA, ARF and AA anti-disease treatment

Parasitized RBC's destruction has minor to insignificant contribution to the pathophysiology of SMA with the disease process driven mainly by increased npRBC's destruction and erythropoietic suppression [148,149]. The parasitaemia seen in humans is far less than that observed in the murine models of SMA [ $\leq 4\%$  compared to 80%] for it to drive SMA [150]. The major mechanisms accentuating SMA seem to be controlled by the innate and acquired immune responses with the inflammatory mediators forming the bedrock of the syndrome.

Uninfected red blood cells are tagged for intravascular haemolysis or reticuloendothelial (RES) destruction mediated by a number of mechanisms. These include: RBC membrane oxidation, RBC membrane fluidity changes, unregulated complement binding on RBC's, autoantibody binding and immune complex formation [151-156]. The immunological nature of the RBC tagging systems is apparent and the pathogen associated membrane proteins (PAMPs) such as haemozoin and GPI are at the core of this process [157-163]. RBC targeting for destruction are parasite induced events, but the process effectors are independent from the initial stimuli as SMA may continue with hyper-activated RES and hyper-splenism centrally orchestrating an inflammatory mandate [164,165]. This may imply that phytochemicals with anti-inflammatory, antioxidant and immunomodulatory activities, like AA, may have a significant anti-disease impact on SMA development and ARF through inhibition of the soluble disease mediators as well as the release of PAMPs.

Red cell membrane fluidity changes are most common mechanism by which cell destruction is instituted through shortened RBC's survival. Inhibition of the erythrocyte magnesium-activated ATPase is a common feature in falciparum infection which is driven by the NO and ONOO<sup>-</sup> that changes membrane fluidity leading to haemolysis [166]. Furthermore, the calmodulin-dependent erythrocyte kinetics are altered by secondary calcium influx when ATPase pump fails, resulting in haemoglobin-cell membrane interaction reduction and curtailing deformability and increasing mechanical fragility [124]. Invariably, malaria hypovolaemia ensues with possible reduced renal perfusion and ARF. AA may alleviate the ensuing sick-cell syndrome and pseudohypoaldosteronism by inhibition of iNOS and quenching NO thereby reactivating ATPase and ENaC, restoring membrane fluidity and stemming haemolysis.

## Erythropoietic suppression in SMA, ARF and AA administration

Hypovolaemia resulting from SMA, leading possibly to ARF, involve erythropoietic suppression reducing RBC volume emanating from inflammatory components mainly TNF, INF- $\gamma$ , NO, OONO<sup>-</sup>. Indeed, microarray analysis of the spleen and the bone marrow has shown that early *P. berghei* ANKA infection reduces reticulocytosis [167]. Circulatory shock syndrome (algid malaria), which may be associated with ARF in malaria, was ameliorated when immune system related CD8 (+)-T cells were depleted in *P. berghei*-infected mice showing possible immunological derangements which may be alleviated by AA treatment [168].

## Fluid loss in ARF and AA treatment

Hyperpyrexia induced severe sweating and vomiting is common in malarial infection and the anorexia accompanying malaria may decrease fluid intake resulting in dehydration leading to pre-renal ARF [169,170]. All phenomena have inflammatory responses as their basis and may respond well to treatment with an anti-inflammatory like AA. Indeed, malaria treatment with MA or OA tended to reduce variance of food and water intake compared to treated non-infected controls (NIC) but varied significantly with the non-treated infected control (IC) [90,134].

ARF of malarial fluid deficit has an inflammatory element driving the aetiological process, a factor that may need to be exploited in the treatment of the disease. However, common practice of using non-steroidal anti-inflammatories drugs (NSAIDs) may prolong TNF- $\alpha$  synthesis exacerbating the disease which may precipitate pre-renal azotaemia to ischaemic ARF [15,170]. The triterpenes AA becomes a better alternative as an anti-inflammatory agent. Traditionally, AA has been used for kidney disease treatment as a diuretic alleviating oliguric states a situation for which adrenaline and dopamine inotropic infusion have been reported to induce hyperlactaemia and nRA [13,171].

## Cerebral Malaria and AA administration

Cerebral malaria may be defined as a state of unconsciousness characterized by a non-response to localized stimuli and unarousable coma in the absence of hypoglycaemia or pyogenic meningitis, with varying neurological disturbances [172,173]. There is a morbid association of CM with high mortality and death, but without the complications of ARDS and nRA there are far less fatalities of coma indicating a common underlying phenomenon of multi-organ failure of malaria [58]. The possibility of intracranial pressure associated with CM due to cranial arteries obstruction is high although a similar condition with seizures and unconsciousness can also be observed in sepsis where mechanical impediments to blood flow are not present [174]. Intracranial pressure is usually much lower than the systemic circulation and does not vacillate with changes in global blood pressure. The architecture of the cerebral arterioles and capillaries differs with those of the general circulation having comparably thinner walls, less smooth muscles and adventitia providing considerable vascular resistance under the lower pressure in which they function [175]. This makes these vessels more vulnerable to vasodilation if iNOS induction would occur in them resulting in cerebrovascular accidents than increased intracranial pressure noted in malaria. Reduced oxygen utilization leading to coma without sequestration has been observed in septicemia-induced encephalopathy where systemic metabolic effects are determinants of consciousness state [176].

Through the ability to inhibit the Na<sup>+</sup>/K<sup>+</sup> ATPase pump, NO and ONOO<sup>-</sup> from inflammatory responses, may cause accumulation of Na<sup>+</sup> and water (H<sub>2</sub>O) in the brain resulting in brain oedema [52,53]. Brain oedema may lead to poor oxygen supply resulting in coma of CM. In cases of microhaemorrhages or ring haemorrhages seen in malaria, the release of GPI may induce a strong local stimuli overriding constitutive mechanism suppressing the production of iNOS resulting in vasodilation and rupture of the fragile arterioles and capillaries. Glucocorticoids inhibit iNOS over-expression in the absence of macrophage inhibitory factor (MIF), which is elevated in malaria, protecting against cerebral vasodilation [177]. By inhibiting iNOS over expression as seen in  $\lambda$ -carrageenan induced inflammation, AA may ameliorate CM associated vasodilation [59]. Administration of AA was shown to attenuate glutamate-induced cognitive deficits in mice, ceramide-induced neuronal apoptosis and neuroprotection in mouse model focal cerebral ischaemia [20,178,179]. This points to possibilities of AA being able to cross the blood brain barrier (BBB) which implications may allude to the potency of the drug alleviating CM.

The malarial parasite var gene product PfEMP-1 receptors in the brain are over represented by CD36 than either ICAM-1 or VCAM-1 or CSA which are up-regulated by pro-inflammatory cytokines such as TNF- $\alpha$  [180-185]. This reduces incidences of pRBC's agglutination, cytoadhesion and sequestration relative to other organs whilst increasing nonopsonic phagocytosis of pRBC's [186]. Moreover, accumulation of leukocytes and thrombocytes seem to be present in the brain during CM [187,188]. Depletion of activated Th1 is known to alleviate murine CM [149]. AA induces apoptosis of activated lymphocytes and macrophages, accelerate neurite elongation and nerve regeneration, aspects essential in CM resolution and cognitive restoration [107,189]. AA has also been reported to have neuroprotective properties in mouse model of focal cerebral ischaemia showing possible ability to cross the blood brain barrier which could be beneficial in cerebral malaria [190].

## Conclusion

The pathophysiology of malaria revolves upon three main pillars of RBC's changes as a result of the parasitic infection, immunological host response and the metabolic derangements that follow. The underlying common theme that can be observed in the aetiology of malaria disease is the inflammatory milieu that seem to be seen in all three syndromes intricately intertwining complex events, processes and systems to bring about the malaria disease.

AA is a secondary plant metabolites that bear constitutive antioxidant and oxidative properties that may inhibit growth of parasites and host inflammasome. AA is highly bound to albumin, and may have a prolonged bioavailability period allowing for a longer parasite-drug contact. Selective apoptotic events on activated Th1 and macrophages, neurite elongation and nerve regenerative and metabolic disturbances amelioration of AA persuades us to perceive the phytochemical's influence on malaria. Overall, AA renders itself amenable as a possible anti-parasitic, anti-inflammatory, antioxidant, immunomodulatory, renoprotective, neuroprotective and metabolic elixir of malaria disease.

## Way forward

Much work is required with determining the pharmacokinetics of AA in malaria. Abilities of AA to down-regulate leucocytosis processes and inhibition of iNOS and other molecules vital to the malarial disease need to be explored. The anti-parasitic and anti-disease influence of AA in combination therapy remains a grey area. Posology and efficacy of AA amongst different *Plasmodium* species and strains remains to be determined. Alternative administration methods, like transdermal delivery with the aim of reducing the amount of drug administered and treatment period, may need to be explored further with AA. The ADMET properties of AA in malaria requires investigation.

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## Conflict of interests

The authors declare have no financial or non-financial competing interests in the publication of the manuscript

## Authors' contributions

GAM pioneered conception, design, acquisition of literature, analysis and interpretation of information, drafting the manuscript, revising and approving final copy for submission and is corresponding author; IK revised manuscript draft and added critical intellectual content and accountability to final work.

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