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RESEARCH ARTICLE

Medicinal plants with antimalarial activities mediated via glycogen synthase kinase-3 beta (GSK3β) inhibition

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ABSTRACT

Many of the therapeutic effects of plant extracts and bioactive compounds appear related to their immunomodulatory effects and impact on the host immune system. The immune response is desirable to mitigate established infections and, in the case of severe malaria, is a feasible approach to dealing with the overwhelming cytokine response. Glycogen synthase kinase-3 (GSK3), a Ser/Thr kinase that is a central regulator of the cytokine response, is a promising antimalarial drug target. In this review, we discussed our ongoing research projects, which include assessing the antimalarial activities of medicinal plants and their bioactive compounds, immunomodulatory activities mediated by GSK3, and the potential inflammatory pathway involved in malarial infection.

Keywords: Medicinal plants; kaempferol; curcumin; antimalarial activities; glycogen synthase kinase-3 beta.

INTRODUCTION

Modulation of immune responses (immunomodulation) using medicinal plants and their products has emerged as a potential effective therapeutic strategy (Chouhan *et al*., 2014) in inflammation-related diseases. Many of the therapeutic effects of plant extracts and bioactive compounds appear to be associated to their immunomodulatory effects on the host immune system (Jantan *et al*., 2015). Augmentation of the immune response is desirable to mitigate established infections, and in the case of severe malaria, is a feasible approach to address the overwhelming cytokine response. Protein kinases are recognised as potential therapeutic targets for malaria (Nag *et al*., 2013). Glycogen synthase kinase-3 (GSK3), a Ser/Thr kinase which is a central regulator of the cytokine response represents a viable candidate as an antimalarial drug target. Inhibition of GSK3β is potentially useful to modulate the cytokine imbalance during infection (Cortés-Vieyra *et al*., 2012).

Malaria may be categorised as an inflammation-related disease on the basis of the similarities in inflammatory responses elicited by the plasmodial parasites as in other pathogenic infections. In cases where parasite elimination cannot be achieved through antiparasitic approaches, immunomodulatory strategies may be considered as adjunctive therapy to reduce disease severity and burden. Many medicinal plants and their products have been screened for their cytokine-modulating effects mainly to address the altered balance of these inflammatory molecules during infection. This review covers our on-going research initiatives which involve evaluations of antimalarial activities of medicinal plants and their bioactive compounds with immunomodulatory activities mediated through GSK3. We focus our investigations on medicinal plants (*Gynura procumbens, Gleichenia truncata*, *Curcuma longa* and *Andrographis paniculata*) and bioactive compounds (kaempferol, quercetin, methyl-4-hydroxycinnamate, curcumin and andrographolide) to evaluate their GSK3-inhibitory effects in a rodent malarial infection model.

GSK3 as a drug target for malaria

Malaria is second to tuberculosis as the leading cause of morbidity and mortality as a consequence of a single infectious disease (Lacerda-Queiroz *et al*., 2011). Much effort has been taken to diminish the disease which affected 241 million people and caused approximately 627 000 deaths in 2020 (WHO, 2021). Taking into account rapid development of resistance to front-line drugs (e.g., chloroquine and artemisinin) and the emergence of zoonotic *Plasmodium knowlesi* infection, there is an urgent need for more effective therapeutics including those associated with novel modes of action (Li *et al*., 2016). Much of the pathology of malaria is exacerbated by the host inappropriate or excessive immune response in an attempt to eliminate the parasite (Lacerda-Queiroz *et al*., 2011). Multiple cytokine responses are induced during malarial infections. High production of inflammatory cytokines or 'cytokine storm' are detected in the bloodstream of malaria patients (Clark *et al*., 2008). As a consequence of increased inflammatory cytokine generation such as TNF-α, IL-1 and IL-6, harmful sequestration of

parasitised red blood cell can be viewed quantitatively from post-mortem microscopy of the microvascular of cerebral tissue (Ponsford *et al*., 2012). The consequence of excessive cytokine production is also associated with other pathogenesis such as acute respiratory distress syndrome and multiple-organ failure (Clark *et al*., 2008; Dunst *et al*., 2017). In rodent malarial infection, *Plasmodium berghei* NK65 parasite infection in ICR mice resulted in increased in IL12-p40 (Yoshimoto *et al*., 1998) and IL-18 (Adachi *et al*., 2001) levels. In other study, infection of C57BL/6 and BALB/c mice with the NK65 strain of *P. berghei* elevated levels of sera TNF-α and IFN-γ which eventually led to cerebral malaria (CM) (Lacerda-Queiroz *et al*., 2011). Antimalarial drug development efforts are now concentrating not only on antiparasitic effects, but also on immunomodulatory activities in the host (Mimche *et al*., 2011).

Protein kinases are among drug targets that have attracted much attention. Of interest is glycogen synthase kinase-3 (GSK3) (Doerig *et al*., 2008) which is pivotal in the regulation of cytokine response in parasitic and bacterial infections (Wang *et al*., 2011). Glycogen synthase kinase 3 (GSK3) is a highly expressed serine/threonine kinase originally identified and named as a kinase that phosphorylates and inactivates glycogen synthase (GS) (Embi *et al*., 1980). GSK3 has been shown to phosphorylate a wide range of cellular proteins, and is involved in multiple cellular processes (Takahasi-Yanaga, 2013; Beurel *et al*., 2015). Many human diseases have been reported to be associated with dysfunctions of GSK3 (e.g. Alzheimer's disease, type-2 diabetes and cancer) (Eldar-Finkelman, 2002; Wang *et al*., 2011). GSK3 appears to play important roles in the host response to viral (Kehn-Hall *et al*., 2012) and fungal (Spinnler *et al*., 2010) infections as well as parasitic infections, including malaria (Osolodkin *et al*., 2011). In mammals, two highly related GSK3 genes encode for the α and β isoforms of the enzyme (Cross *et al*., 1995) each inhibited by phosphorylation of Ser21 and Ser9 residues respectively. The enzyme is active under basal conditions. The major GSK3β-regulating event is Ser9 phosphorylation (Wang *et al*., 2014). Multiple extracellular signals induce rapid Ser9 phosphorylation and result in decreased GSK3β activity. Among the reported upstream regulator of GSK3β is phosphoinositide 3-kinase (PI3K)-AKT/protein kinase B (PKB) (Cross *et al*., 1997). Furthermore, several other kinases have been shown to phospho-inactivate GSK3, including 90 kDa ribosomal protein S6 kinase 1 (p90RSK), serum and glucocorticoid-regulated kinase 1 (SGK1), and MAPK-p38. Since these kinases are all members of the protein kinase A,G, and C (AGC) family, it is possible that GSK3 will be phospho-inactivated by other members of this large kinase group that regulates a wide range of physiological processes (Beurel *et al*., 2015).

GSK3 is also pertinent in the plasmodial life cycle (Masch & Kunick, 2015). *Plasmodium falciparum* glycogen synthase kinase-3 (*Pf*GSK3) is one of the eukaryotic protein kinases identified as essential for malarial parasite development (Masch & Kunick, 2015). Selective inhibitors of *Pf*GSK3 with direct effects on parasite development have been screened from compound libraries either against cultured parasites or against isolated target molecules validated as essential for parasite viability (Droucheau *et al*., 2004). The diversity of GSK3 functions in the regulation of cellular processes ranging from cell cycle, differentiation to metabolism (Osolodkin *et al*., 2011) led to speculations not only on possible physiological roles of *Pf*GSK3 in malaria (Masch & Kunick, 2015) but also on the immunomodulatory effects of GSK3 on the host.

Several antiplasmodial agents discovered in phenotypic high-throughput screening (HTS) campaigns were previously synthesised as protein kinase inhibitors or resemble known protein kinase inhibitors (Gamo *et al*., 2010). Although protein kinases have previously been proposed as antiplasmodial drug targets (Doerig *et al*., 2008; Zhang *et al*., 2012), much need to be understood before their clinical use. Phenotypic screening efforts for inhibitors of human protein kinases against *Plasmodium* parasites have revealed that inhibitors of human p38 map kinase (Brumlik *et al*., 2011), human cyclin-dependent kinases (Houze *et al*., 2014) and human VEGFR-2 (Hempel *et al*., 2014) were able to inhibit *P. falciparum.* Phenotypic screening of protein kinase inhibitor libraries ultimately led to the development of a compound, imidazopyridazine which exhibited curative activity in *P. berghei*-infected mice (Le Manach *et al*., 2015). Furthermore, an investigation revealed of more than 1000 protein kinase inhibitors that inhibited both liver and blood stages of the malarial parasite. Based on the kinase inhibition characteristics of hit molecules, it was determined that glycogen synthase kinase-3 (GSK3) plays an important role in parasite inhibition (Derbyshire *et al*., 2014).

We previously demonstrated that LiCl, a GSK3 inhibitor, suppressed parasitaemia progression in a rodent malaria infection model and increased animal survivability rate, implying a role for this kinase in malarial infection (Zakaria *et al*., 2010). It should be noted that *in vitro* antiplasmodial and *in vivo* chemosuppressive effects within certain limits are frequently employed in many malarial studies to evaluate antimalarial properties of test extracts or compounds. Outcomes from *in vivo* experimentations however included not only intrinsic antiparasitic (antiplasmodial) effects but also effects on host.

Subsequent to our report on the effects of LiCl on *P. berghei* NK65-infected mice, Dai *et al*. (2012) reported that LiCl treatment restored neuro-cognitive function in murine experimental cerebral malaria (ECM). The current lack of specific therapies aimed at dampening the proinflammatory state associated with the neurologic syndrome, as well as its deleterious effects on the host, is indeed the major challenge in preventing and reducing mortality from malaria (Sahu *et al*., 2015). We have demonstrated that curcumin, a bioactive compound from *C. longa* with reported GSK3-inhibitory properties displayed antimalarial effects involved modulation of cytokine balance (Ali *et al*., 2017).

Das *et al*. (2015) provided preliminary evidence that medicinal plants containing limonin, tangeritin, 6 gingerol, zerombone, and ganoderic acid A may be applied to high-grade meningiomas as a therapeutic agent. Treatment with these compounds against tumor cells resulted in the induction of apoptosis with enhanced phosphorylation of GSK3β via inhibition of the Wnt5/β-catenin pathway. On the other hand, a study on bacterial infections by Wang *et al*. (2014) indicated that Wnt/β-cat pathway may independently lead to phospho-inactivation of GSK3β and stabilisation of β-catenin. Suppression of inflammation occured by interference of NF-κB in a similar manner to IκB (inhibitor of κ light polypeptide gene enhancer in B cells) (Duan *et al*., 2007). Active GSK3β was shown to phosphorylate directly NF-κB p65 (Viatour *et al*., 2004) thus phospho-activating NF-κB p65-driven proinflammatory genes. GSK3 inhibitors can alter the magnitude of the inflammatory response by differentially regulating production of pro and antiinflammatory cytokines. Inhibition of GSK3 either directly or indirectly upon phosphorylation may be able to dampen the proinflammatory cytokine expression thus reducing the effects of the excessive inflammation which usually leads to death.

Medicinal plants with antimalarial activities related with GSK3 inhibition

As in other pathogenic infections, plasmodial parasite invasion of host triggers a series of events leading to inflammatory response within the host to oust the invading microbe (Kaur *et al*., 2009). One of the important pathways involved in this response is the PI3K/AKT pathway (Yoo *et al*., 2005). GSK3, a downstream component of the PI3K/AKT pathway is pivotal in mediating the inflammatory response (Wang *et al*., 2011). Hence, modulating immune response is seen as an alternative approach in combating malaria since mortality cases related to the disease keep increasing caused by the excessive inflammatory response during infection. Due to parasitic resistance development toward front-line drugs, chloroquine and artemisinin and the emergence of *P. knowlesi* zoonotic infection in Malaysia,

screening for bioactive compounds from medicinal plants such as *G. procumbens, G. truncata*, *C. longa,* and *A. paniculata* to develop novel antimalarial therapeutics is pursued.

Gynura procumbens, a medicinal plant belonging to the Astereceae (Compositae) family (locally known as *Sambung Nyawa* in Malaysia), is commonly found growing wild or cultivated in various parts of South-east Asia (Bhore *et al*., 2010). Poultices and boiled extracts from this plant have been used to treat ailments ranging from skin conditions and fevers to kidney disease, inflammation and diabetes (Perry, 1980). We were the first to report active and selective antiplasmodial activities of both aqueous and ethanolic extracts of the plant against cultures of chloroquine-sensitive *P. falciparum* 3D7 (Vejanan *et al*., 2012). When evaluated *in vivo,* repetitive intraperitoneal injections of up to 250 mg/kg/day each of aqueous and ethanolic extracts of *G. procumbens* for four consecutive days into *P. berghei* NK65-infected mice resulted in chemosuppressive effects and improved median survival time implicating good antimalarial activity of the plant. We proceeded to investigate the effects of *G. procumbens* extract administration on the phosphorylation state of liver GSK3β (Wong *et al*., 2015) since we had shown earlier (Zakaria *et al*., 2010) that the GSK3 inhibitor, LiCl was able to suppress parasitaemia development in malaria-infected mice. Our analyses revealed that administration of the aqueous extract of *G. procumbens* resulted in increased (2.3-fold) in liver pGSK3β (Ser9) of *P. berghei*-infected mice. This implies that the antimalarial activity of the *G. procumbens* extract may be associated with inhibition of host GSK3 (Figure 1).

Gleichenia truncata is a high-altitude medicinal fern from the Gleicheniaceae family that has been traditionally used to treat fever among ethnic communities throughout Asia (Jaman & Latiff, 1999; Ho *et al*., 2010). Antibacterial, antiglucosidase and antioxidant effects are among the pharmacological properties reported to be associated with this fern (Chai *et al*., 2013). Intraperitoneal administration of up to 250 mg/kg b.w. of *G. truncata* (crude methanolic extract) suppressed *P. berghei* parasitaemia development by >60% in infected mice (Suhaini *et al*., 2015). Increased Ser9 phosphorylation of liver GSK3β (6-fold) was detected in *P. berghei*-infected animals administered with *G. truncata* extract compared to controls. These antiplasmodial and chemosuppressive effects, improved median survival time and increased Ser9 phosphorylation of GSK3β demonstrate that the antimalarial properties observed with this fern are mediated through inhibition of GSK3β (Sudi, 2015) as seen for *G. procumbens*. The *in vitro* and *in vivo* antimalarial activities of plants extracts with GSK3-inhibitory properties are listed in Table 1.

Curcuma longa or the turmeric extract consists of 60-70% curcumin, 20-27% demethoxycurcumin and 10-15% bisdemethoxycurcumin. Curcumin is a major constituent in the turmeric extract of *C. longa*. The turmeric extract has also been reported to have many beneficial health properties ranging from antimalarial, antiaging, anticancer, antihypertensive,

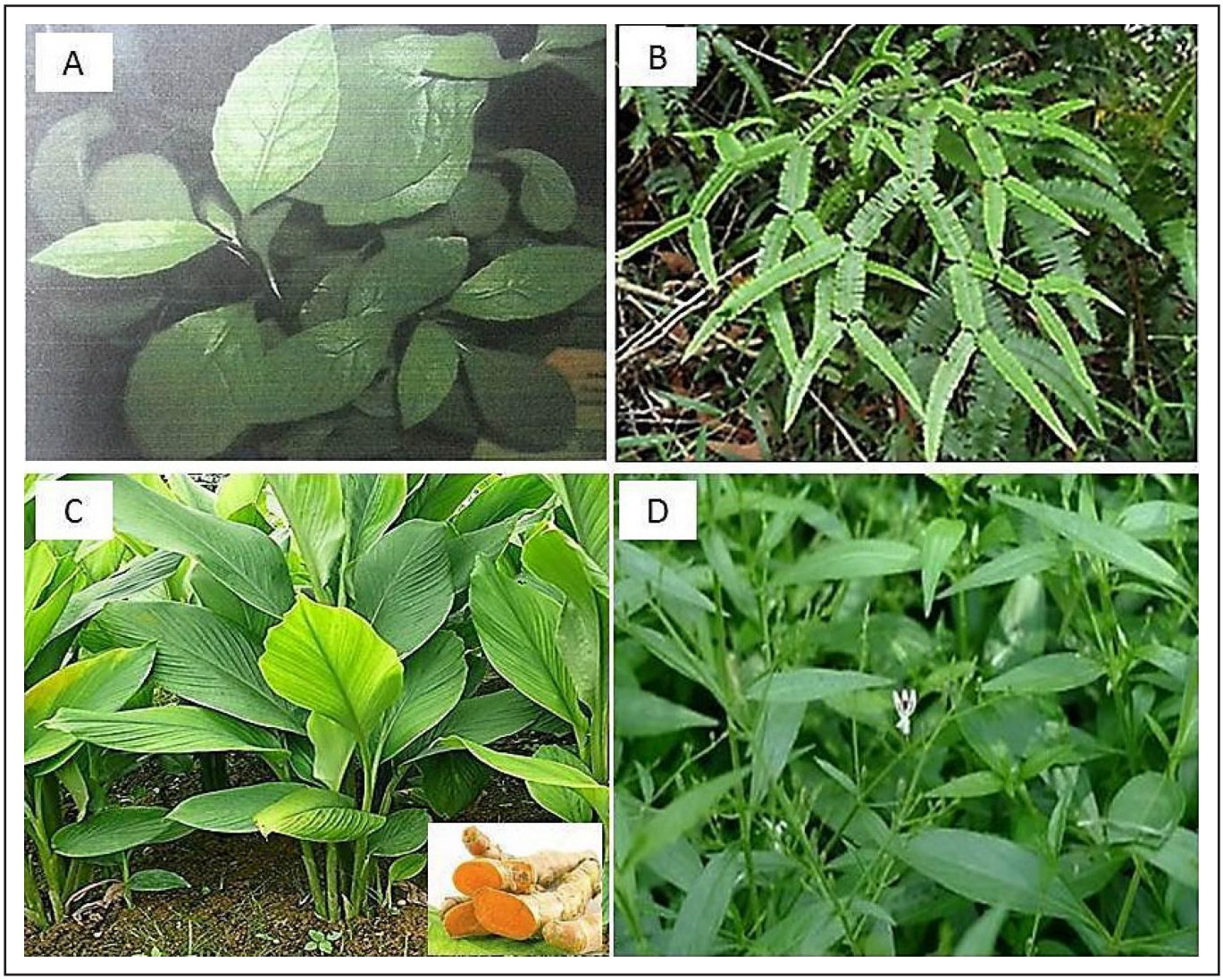

Figure 1. Examples of medicinal plants with antimalarial activities related with GSK3 inhibition; A. *Gynura procumbens*; B. *Gleichenia truncata*; C. *Curcuma longa*; D. *Andrographis paniculata.*

Table 1. Plant extracts associated with GSK3-inhibitory properties

Plants	Pharmacological effects	*In vitro* antiplasmodial activity against *P. falciparum* 3D7 (IC_{50})	*In vivo* antimalarial effects against *P. berghei* NK65 (chemo-suppression percentage)	GSK3 inhibition properties (Western blotting analysis)	References
Gynura procumbens	Antimalarial, antiinflammation, antidiabetic, fever, skin disease	25.69 ± 4.34 µg/mL (aq) IC_{50} 42.23 ± 7.19 µg/mL (EtOH)	65.95 ± 5.48 % (aq) 64.44 ± 4.05 % (EtOH)	Increased liver GSK3β (Ser9) phosphorylation (2.32-fold) in malarial infection	Vejanan, 2014; Wong *et al*., 2015
Gleichenia truncata	Antiinflammation, antidiabetic, fever, skin disease	0.82 ± 0.18 µg/mL (MeOH)	73.68 ± 5.50 % (MeOH)	Increased liver GSK3β (Ser9) phosphorylation (6.00-fold) in malarial infection	Suhaini *et al*., 2015
Curcuma longa (turmeric)	Antimalarial, antiinflammation, antidiabetic, fever, skin disease	<0.625 µg/mL (EtOH)	67.9% (EtOH)	Inhibited GSK3β in malarial infection, and targeted PI3K/ Akt pathway in multiple cancer cells and Alzheimer's disease	Lwin *et al*., 2017; McCubrey *et al*., 2017; Ali *et al*., 2017
Andrographis paniculata	Antimalarial, fever, diarrhoea, cardiovascular disease, and antioxidant	7.2 µg/mL (MeOH)	>60% (MeOH)	Increased levels of inactive p-GSK3β in Alzheimer's disease	Mishra *et al*., 2011; Gu *et al*., 2020

antiinflammatory and antineurological effects. *In vitro* antimalarial activity of the ethanolic extract of *C. longa* Linn. extract showed schizont suppression of 62.63% with IC_{50} values of <0.625µg/mL. *In vivo* parasite suppression exerted 67.9% schizont suppression at the dosage of 50 mg/kg body weight for *C. longa* extract (Lwin *et al*., 2017). Curcumin targets multiple signalling pathways including PI3K/PTEN/Akt/mTORC, and WNT/β-catenin in cancer diseases. Curcumin can also suppress proliferation and induce apoptosis in non-small cell lung cancer (NSCLC) via suppression of the PI3K/PTEN/AKT pathway (McCubrey *et al*., 2017). In malarial infection, we have previously shown the effect of curcumin administration on PI3K/AKT/GSK3 pathway involving activation of AKT (phosphorylation at Ser473) and inhibition of GSK3β (phosphorylation at Ser9) in liver of malarial infected animals (Ali *et al*., 2017).

Andrographis paniculata is a herbal plant with antiinflammatory, antioxidant properties, protecting against Alzheimer's disease (AD) and antimalarial activities (Mishra *et al*., 2011). The major phytoconstituents from *A. paniculata* are andrographolides, which were reported to have an inhibitory effect on *Plasmodium* sp. (Mishra *et al*., 2011) and human hepatic cytochrome P450, alpha-glucosidase and alphaamylase enzymes that cause type 2 diabetes (Subramanian *et al*., 2008). *A. paniculata* extract exerted a promising antimalarial activity, IC_{50} of 7.2 µg/mL and the extract inhibited the ring stage of the parasite and exerted >60% chemosuppression on *P. berghei* growth (Mishra *et al*., 2011). *A. paniculata* extract also provided convincing evidence that the neuroprotective effects were partially related to APP-BACE1-GSK3β signalling pathway in inflammatory response (Gu *et al*., 2020).

Bioactive compounds of plant-origin with antimalarial activities related with GSK3 inhibition

In addition to the antimalarial studies of medicinal plants as described above, we extended our study to investigate the activities of bioactive compounds found in *G. procumbens, G. truncata*, *C. longa* and *A. paniculata* on the modulation of inflammatory response in *P. berghei*-infected mice. *In vitro* antiplasmodial activity of kaempferol (identified from *G. procumbens*) showed moderate activity with an IC_{50} of 30.94 ± 1.48 µM against 3D7 strain of *P. falciparum*. Post-infection treatment (therapeutic treatment) with 5 mg/kg b.w. of kaempferol exerted strong chemosuppressive activities and prolonged survivability in infected animals, hence indicating good antimalarial activity of the compound (Wong *et al*., 2015). Interestingly kaempferol, a bioactive compound identified in *G. procumbens* (Akowuah *et al*., 2002; Chong *et al*., 2012) was also shown to display antiplasmodial and chemosuppressive effects (Wong *et al*., 2015) thus suggesting that the antimalarial property of *G. procumbens* could be attributed in part to the presence of this compound. Post-infection treatment with kaempferol resulted in a significant decrease (2.0-fold) in serum TNF-α whilst IL-10 and IL-4 were elevated (1.6-fold and 3.4-fold respectively) (Wong *et al*., 2015).

Pre-infection treatment (prophylactic treatment) with kaempferol also resulted in promising chemosuppressive effect and prolonged survivability of infected animals at 20 mg/kg b.w. of kaempferol treatment. Specifically, pre-infection treatment with 5, 10 and 20 mg/kg b.w. kaempferol resulted in chemosuppression of 45.17 ± 6.09%, 19.53 ± 3.56% and 60.33 ± 4.50 % respectively while median survival time increased from 12 to 18 days as compared to control. Western and densitometric analyses showed that pre-infection kaempferol treatment of infected mice showed in increment of GSK3β (Ser9) (2.7-fold) phosphorylation. Pre-infection kaempferol treatment resulted in similar results as described earlier in post-infection treatment with the same compound except that pre-infection treatment required higher concentration of compounds in order to obtain good chemosuppressive effects exceeding 60%. Analyses of cytokine-modulating effects of both pre- and post-infection treated mice revealed elevation and decrement of respective cytokines as compared to control. Kaempferol treatment was found to decrease the level of proinflammatory cytokines (except for IFN-γ in pre-infection treatment) and increased the antiinflammatory cytokines as compared to control (Hassan, 2019b). Besides that, pre-infection treatment with kaempferol caused a significant decrease in TNF-α (6.6-fold) and a significant increase in IL-4 (2.2 fold) in serum. Antimalarial and cytokine-modulating activities of kaempferol as seen from these pre-infection treatment studies thus in part are mediated through inhibition of GSK3β (Hassan, 2019b) similar to that observed in post-infection treatment with kaempferol (Wong *et al*., 2015).

Quercetin is also one of the flavonoid compounds identified in *G. procumbens*. It exhibited moderate antiplasmodial activity *in vitro* with an IC_{50} value of 19.3 µM against the 3D7 strain of

P. falciparum. Quercetin inhibited parasitaemia progression and extended survivability of animals infected with NK65 and ANKA strains. Animals infected with *P. berghei* NK65 and ANKA treated with quercetin (25mg/kg b.w.) showed the highest chemo-suppression of 60.7% and 36.1% ($p<0.05$) respectively. Furthermore, animals infected with NK65 and ANKA strains administered with quercetin respectively displayed increment in pGSK3 (Ser9) at 2.3-fold and 1.2-fold respectively. In addition to that, quercetin also modulated cytokines production in rodent malarial infection. Upon quercetin treatment, the proinflammatory cytokines, TNF-α and IFN-γ were reduced to 6.6- and 2.5-fold, whilst the antiinflammatory cytokines, IL-10 and IL-4 were elevated by 2.1- and 5.7-fold. Findings from the studies indicated that quercetin modulated the inflammatory cytokines via inhibition of GSK3β in the liver of malarial infected animals (Ali *et al*., 2021).

Further evaluations with methyl-4-hydroxycinnamate, one of the bioactive compounds identified in *G. truncata* revealed good *in vitro* antiplasmodial and good chemosuppressive activity with 30 mg/kg b.w. treatment of this compound (Sudi *et al*., 2018). Increased phosphorylation of liver GSK3β was detected in *P. berghei*-infected animals administered with methyl-4-hydroxycinnamate compared to controls. Further investigation on antiinflammatory cytokine response also showed that *P. berghei*-infected animals proinflammatory cytokines (TNF-α and IFN-γ) were lowered and antiinflammatory cytokines (IL-4 and IL-10) were elevated when treated with this compound (Sudi *et al*., 2018). These results demonstrated that the antimalarial properties and cytokine-modulating effects observed with methyl-4-hydroxycinnamate are mediated through inhibition of GSK3β. In addition, a study by Vo *et al*. (2014) revealed that methyl p-hydroxycinnamate exerted antiinflammatory activity through the activation of Akt pathway in LPS-stimulated RAW264.7 macrophage cells.

Curcumin, one of the major bioactive compounds present in *C. longa* exhibits a myriad of bioactivities including antiinflammatory effects in diseases such as malaria (Reddy *et al*., 2005; Cui *et al*., 2007), Alzheimer's disease (Mishra & Palanivelu, 2008) and diabetes (Babu & Srinivasan, 1997). Combination treatments of curcumin with antimalarial front-line drugs have been shown to lower parasitaemia development and improve *P. berghei*-infected animal survivability (Neto *et al*., 2013). Curcumin has also been reported to affect various cell types of the immune system (Jagetia & Aggarwal, 2007). In addition, this compound has been shown to display both direct antiplasmodial and immunomodulatory effects (Mimche *et al*., 2011). However, we were the first to report the involvement of GSK3β in the antimalarial and antiinflammatory effects of curcumin as tested in rodent models of *P. berghei* NK65 (Ali *et al*., 2017) and *B. pseudomallei* (Tan *et al*., 2017). Findings from the aforementioned study revealed strong and selective antiplasmodial activity of curcumin against *P. falciparum* 3D7. Intraperitoneal administration of curcumin into infected animals caused in dose-dependent chemosuppressive effect. At 30 mg/kg b.w., therapeutic and prophylactic administrations of curcumin displayed chemosuppression exceeding 50%, and prolonged animal survivability. Most importantly in relation to GSK3, western analysis revealed a 5.5-fold (post-infection group) and 1.8-fold (pre-infection group) increase in liver pGSK3β (Ser9) of curcumin-treated infected animals. This increase in Ser9 phosphorylation of liver GSK3β with curcumin administration indicated that the chemosuppressive effects of the compound against *P. berghei* infection in mice is associated with inhibition of the host kinase. Furthermore, the inhibition of GSK3β was shown in the same study, to be accompanied by activation of Akt (as seen from increase in Ser473 phosphorylation of Akt in liver of treated infected mice). Curcumin treatment caused a significant decrease (9.0-fold) in serum TNF-α whilst IL-10 was elevated (1.3-fold). These results demonstrate that the antimalarial properties and cytokine-modulating effects observed with curcumin are mediated through inhibition of GSK3β.

Andrographolide, one of the bioactive compounds identified in *A. paniculata*, also showed promising results as a plant-origin immunomodulator in malaria-infected animals. This compound is a potent activator of Wnt signaling and inhibits GSK3β by a non-ATP competitive mechanism (Tapia-Rojas *et al*., 2014). Studies in our laboratory (Hassan *et al*., 2019a) show that andrographolide has good antiplasmodial activity (IC_{50} = 13.7 ± 0.86 μM). Andrographolide treatment resulted in significant chemosuppression (>60%) and prolonged survivability of *P. berghei*-infected mice. Western analysis showed 6.4-fold increase in liver pGSK3β (Ser9) of *P. berghei*-infected mice treated with an effective dosage of andrographolide (5 mg/kg b.w.). Treatment with andrographolide resulted in significant decrease of proinflammatory cytokine (IFN-γ) and increase of antiinflammatory cytokines (IL-4 and IL-10) compared to non-treated control (Figure 2; Table 2).

Treatment with bioactive compounds (kaempferol, quercetin, methyl-4-hydroxycinnamate, curcumin and andrographolide) each not only resulted in chemosuppression and prolonged survivability of plasmodial infected animals but also simultaneously caused suppression of GSK3β and modulated the levels of pro and antiinflammatory cytokines. It is noteworthy that our results are similar with that from previously reported studies where administration of extracts and compounds altered proinflammatory and antiinflammatory cytokines. Suppression of GSK3 in *S. typhimurium* (Duan *et al*., 2007) and *F. tularensis* infections (Zhang *et al*., 2009) reduced proinflammatory cytokine production whilst elevating production of antiinflammatory cytokines. Findings from all the studies pertaining to GSK3 phosphorylation indicated that pharmacological activities of extracts and bioactive compounds with respect to antimalarial and antiinflammatory effects are mediated through inhibition of GSK3.

GSK3β inflammatory pathway in malarial infection

Although the function of GSK3β in the inflammatory response during parasite infection has been studied, however more studies are necessary to get a deeper insight into the involvement of GSK3β in inflammatory pathway of malarial infection. From our findings in malarial infection, *P. berghei* NK65 induced excessive inflammatory response through GSK3β in the liver of infected animals and increased production of inflammatory cytokines TNF-α, IFN-γ, IL-10, and IL-4 production during infection (Ali *et al*., 2017; Hassan *et al*., 2019a; Ali *et al*., 2021). In other studies, inflammatory response via GSK3β in parasites has been identified in *Leishmania donovani* infection. In the *L. donovani* infection in RAW264.7 murine macrophages and bone marrow-derived monocytes, GSK3β is phosphorylated and inhibited by AKT. Thus, GSK3β is unable to phosphorylate β-catenin and regulates the activation of a proapoptotic transcriptional regulator, forkhead box

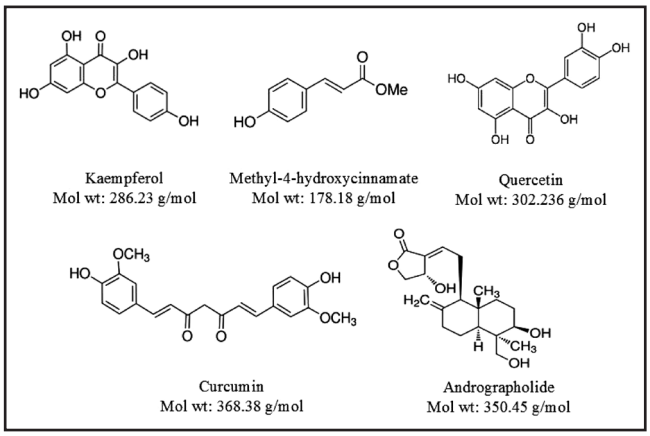

Figure 2. Bioactive compounds from medicinal plants related to GSK3 properties in malarial infection.

Table 2. Bioactive compounds with GSK3-inhibitory properties in malarial infection

Bioactive compounds	Properties	*In vitro* antiplasmodial activity against *P. falciparum* 3D7 (IC_{50})	*In vivo* antimalarial effects against *P. berghei* NK65 (chemo-suppression percentage)	GSK3 inhibition properties (Western blotting analysis)	Cytokine modulating effect	References
Kaempferol	Antimalarial, antiinflammation, antidiabetic, antioxidant, cardioprotective effect, reduced risk of pancreatic cancer	30.94 ± 1.48 µM	60.27 ± 3.20 % (5 mg/kg; therapeutic treatment)	Increased liver GSK3β (Ser9) phosphorylation (2.05-fold; therapeutic treatment)	↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)	Wong *et al.*, 2015
			60.33 ± 4.50 % (20 mg/kg; prophylactic treatment)	Increased liver GSK3β (Ser9) phosphorylation (2.7-fold; prophylactic treatment)	↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (prophylactic)	Hassan, 2019b
Methyl-4-hydroxycinnamate	Antimalarial, antiinflammation, antifungal	8.41 ± 1.25 µM	68.44 ± 8.29 % (30 mg/kg; therapeutic treatment) 78.35 ± 8.13 % (60 mg/kg; prophylactic treatment)	Increased liver GSK3β (Ser9) phosphorylation (7.00-fold; therapeutic treatment)	↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)	Sudi *et al.*, 2018; Hassan, 2019b
Curcumin	Antimalarial, antiinflammation, antidiabetic, fever, skin disease, antibacterial, antiviral	4.34 ± 1.59 µM	67.61 ± 1.71 % (30 mg/kg; therapeutic treatment)	Increased liver GSK3β (Ser9) phosphorylation (5.50-fold; therapeutic treatment)	↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)	Ali *et al.*, 2017
			57.40 ± 1.33 % (30 mg/kg; prophylactic treatment)	Increased liver GSK3β (Ser9) phosphorylation (1.8-fold; prophylactic treatment)	↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (prophylactic)	Ali *et al.,* 2017
Andrographolide	Antimalarial, antiinflammation, antifungal	13.7 ± 0.86 µM	60.17 ± 2.12 % (5 mg/kg; therapeutic treatment) 60.82 ± 6.69 % (15 mg/kg; prophylactic treatment)	Increased liver GSK3β (Ser9) phosphorylation (6.40-fold; therapeutic treatment)	↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)	Hassan *et al.*, 2019a
Quercetin	Antimalarial, antiinflammation	19.31 ± 1.26 µM	67.61 ± 1.71 % (30 mg/kg; therapeutic treatment) 57.40 ± 1.33 % (30 mg/kg; prophylactic treatment)	Increased liver and brain GSK3β (Ser9) phosphorylation (2.30-fold and 1.2-fold respectively; therapeutic treatment)	↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)	Ali *et al.,* 2021; Hassan, 2019b

protein O1 (FOXO-1), by limiting both proinflammatory response and macrophage apoptosis. Macrophages infected with *L. donovani* with an active GSK3β mutant showed a reduction in parasite growth, low expression of IL-10, and an increase in IL-12 production (Gupta *et al*., 2016). In a similar situation, GSK3β inhibition reduced the inflammatory response in induced sepsis animal model (Dugo *et al*., 2007).

Plant bioactive compounds are able to inhibit the excessive inflammatory response via GSK3β phosphorylation. In this review, our on-going research has demonstrated four plants and five bioactive compounds that inhibited inflammatory response via GSK3β phosphorylation at Ser9. Based on our findings, PI3K/AKT inflammatory pathway is involved in the inhibition of GSK3β induced by bioactive compounds (kaempferol, quercetin, methyl-4-hydroxycinnamate, curcumin, and andrographolide). Upon the activation of TLR/PI3K/AKT/GSK3β signalling by malarial parasite or stimuli, immunomodulators or bioactive compounds reduced the inflammation through modulation of GSK3β activity (Ali *et al*., 2017; Ali *et al*., 2021). The immunomodulators can control the inflammation by a decrement in GSK3β activity or phosphorylation at Ser9 which cause GSK3β inhibition. GSK3β inhibition will later induce inhibition of the NF-κB and the activation of β-catenin, CREB, AP1, and STAT1/3. This action will cause a reduction in the expression of proinflammatory cytokines (TNF-α and IFN-γ), and an increase in the antiinflammatory cytokines (IL-10 and IL-4). GSK3β is also inhibited or phosphorylated by S6K, PKA/C, and Dvl3 proteins. Activation of the canonical Wnt signalling pathway caused activation of GSK3β, thus inducing β-catenin degradation and NF-κB activation. The active state of phosphorylated GSK3β at Tyr216 caused the activation of NF-κB and the inhibition of AP1, CREB, STAT1/3, and β-catenin, and later it induced the production of proinflammatory cytokines (Cortés-Vieyra *et al*., 2012) (Figure 3).

Other studies have also demonstrated the potential of plant-derived compounds as immunomodulators via GSK3β inhibition not specifically in malarial infection. In another *P. berghei* infection study, anthocyanins exerted antiinflammatory activity and reduced the inflammatory effect induced by LPS stimulation through an increment in pGSK3β (Ser9) (Khan *et al*., 2019). Apigenin, a flavonoid from *Matricaria chamomilla* inhibited the production of LPS-induced cytokines, TNF-α, IL-1β, and IL-6 via activation of the GSK3β/Nrf2 signalling pathway and suppression of NF-κB activation in BV2 microglia (Chen *et al*., 2020). Similarly, gastrodin, a phenolic compound from *Gastrodia elata*, mediated antiinflammatory and antiproliferation effects in LPS-stimulated by modulating the Wnt/GSK3β/β-catenin signaling pathway in BV-2 microglia (Yao *et al*., 2019). Compounds such as trigonoreidon B identified from *Rigonostemon reidioides*, betulin from the bark of birch trees, and xanthohumol from *Humululus lupulus* induced inhibition of the inflammatory effect by inhibition of pGSK3β (Ser9) in BV2, RAW 264.7 cells, and animal lungs (Luo *et al*., 2015; Ci *et al*., 2017; Lv *et al*., 2017; Utaipan *et al*., 2018).

Taken together, findings from the malarial infection studies described above suggest that the underlying mechanism of the antimalarial and immunomodulatory actions of plant extracts and bioactive compounds in a murine model of malarial infection involved inhibition of host GSK3β and the potential inflammatory pathway involved. The above-mentioned extracts and bioactive compounds are therefore potential immunomodulators of plant-origin for adjunctive therapy against malaria.

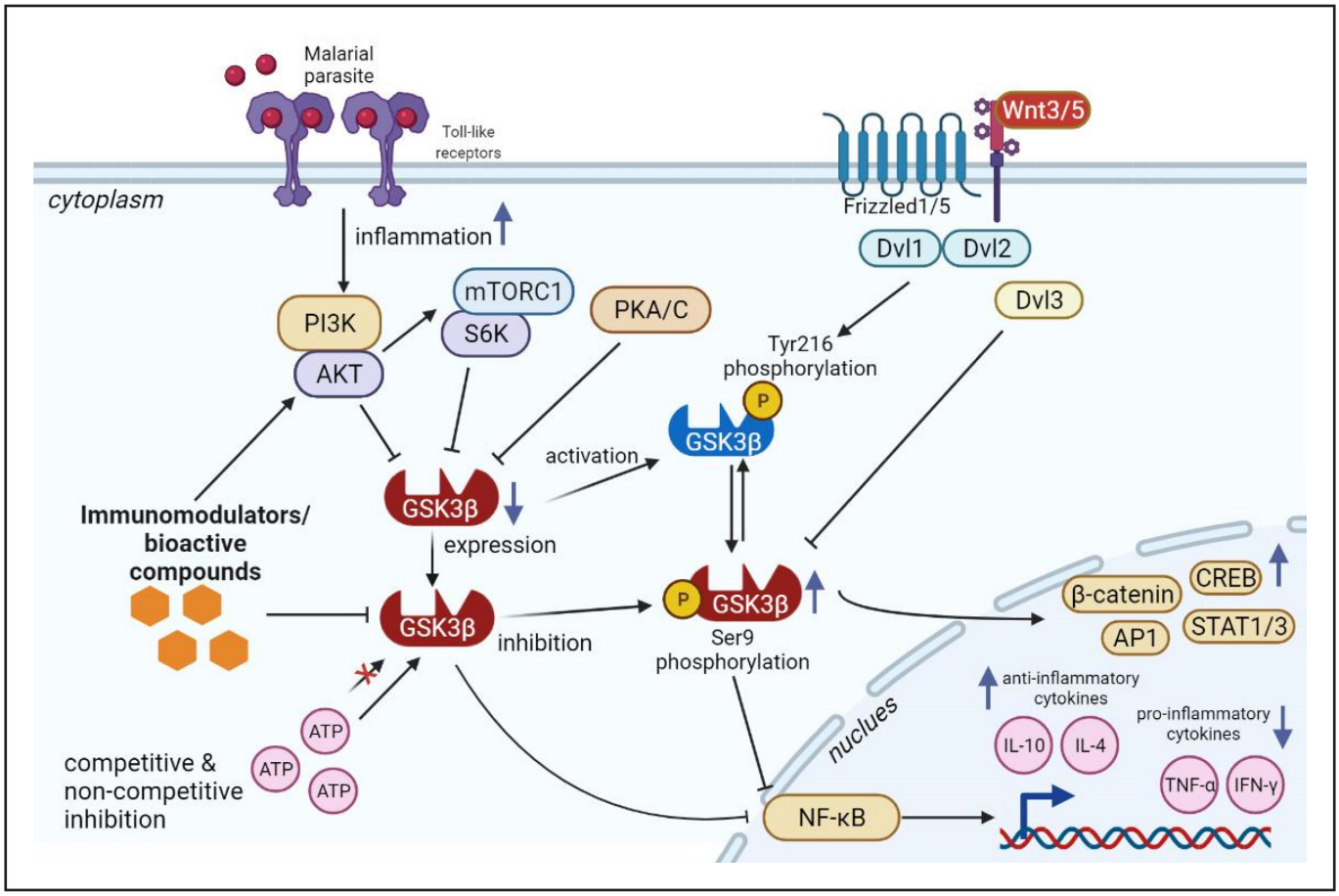

Figure 3. GSK3β inflammatory pathway induced by immunomodulators or bioactive compounds in malarial infection.

CONCLUSION

In conclusion, our data adds to the growing list of plant-based compounds and medicinal plants that exhibit pharmacological activity via inhibition of GSK3. It is now evident that GSK3β is an important target of plant-derived bioactive compounds. Thus plant-derived immunomodulators involving GSK3β are plausible adjunctive therapeutics for inflammation-related conditions. In addition, we have also provided scientific evidence for the use of these medicinal plant (*G. procumbens*, *G. truncata, C. longa* and *A. paniculata*) and plant derived compounds (kaempferol, quercetin, methyl-4-hydroxycinnamate, curcumin, and andrographolide) as remedy for inflammation-related conditions.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviation

AP-1:	activator protein 1
CREB:	cAMP-response element binding protein
Dvl1/2/3:	Dishevelled segment polarity protein 1, 2 and 3
GSK3β:	glycogen synthase kinase 3 beta
NF-κB:	nuclear factor kappa-light-chain-enhancer of activated B cells
PI3K:	phosphoinositide 3-kinase
PKA/C:	protein kinase A/C
PKB:	protein kinase B, also known as Akt
STAT1-3:	signal transducers and activators of transcription 1-3
S6K:	ribosomal protein 6 kinase
TLR:	Toll-like receptor
TNF-α:	tumor necrosis factor alpha
Wnt:	Wingless-related integration site member

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