RESEARCH ARTICLE

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


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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 related 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, 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.

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 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; Durst 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 virion (Kehn-Hall et al., 2012a and b) 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 phosphorylate 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 (GSK3) is one of the eukaryotic protein kinases identified as essential for malarial parasite development (Masch & Kunick, 2015). Selective inhibitors of PfGSK3 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 (Drouicheau 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 PfGSK3 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 (Hemipel 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, tangerin, 6-gingerol, zerumbone, 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 Wnt/β-catenin pathway. On the other hand, a study on bacterial infections by Wang et al. (2014) indicated that Wnt/β-catenin pathway may independently lead to phospho-inactivation of GSK3β and stabilisation of β-catenin. Suppression of inflammation occurred by interference of NF-κB in a similar manner to 1kβ (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 Asteraceae (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,
antinflammatory 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 Ser9) in liver of malarial infected animals (Ali et al., 2017).

*Andrographis paniculata* is a herbal plant with antinflammatory, antioxidant properties, protecting against Alzheimer’s disease (AD) and antimalarial activities (Mishra et al., 2011). The major phytoconstituents from *A. paniculata* are androgapholides, 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 antinflammatory 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

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**Table 1. Plant extracts associated with GSK3-inhibitory properties**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Pharmacological effects</th>
<th>In vitro antiplasmodial activity against <em>P. falciparum</em> 3D7 (IC_{50})</th>
<th>In vivo antimalarial effects against <em>P. berghei</em> NK65 (chemo-suppression percentage)</th>
<th>GSK3 inhibition (Western blotting analysis)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gynura procumbens</em></td>
<td>Antimalarial, antinflammation, anti diabetic, fever, skin disease</td>
<td>25.69 ± 4.34 µg/mL (aq) IC_{50} 42.23 ± 7.19 µg/mL (EtOH)</td>
<td>Increased liver GSK3β (Ser9) phosphorylation (3.2-fold) in malarial infection</td>
<td>Vejanan, 2014; Wong et al., 2015</td>
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<tr>
<td><em>Gleichenia truncata</em></td>
<td>Antinflammation, anti diabetic, fever, skin disease</td>
<td>0.82 ± 0.18 µg/mL (MeOH)</td>
<td>Increased liver GSK3β (Ser9) phosphorylation (6.00-fold) in malarial infection</td>
<td>Suhaini et al., 2015</td>
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<tr>
<td><em>Curcuma longa</em> (turmeric)</td>
<td>Antimalarial, anti diabetic, fever, skin disease</td>
<td>&lt;0.625 µg/mL (EtOH)</td>
<td>Inhibited GSK3β in malarial infection, and targeted PI3K/Akt pathway in multiple cancer cells and Alzheimer’s disease</td>
<td>Lwin et al., 2017; McCubrey et al., 2017; Ali et al., 2017</td>
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<tr>
<td><em>Andrographis paniculata</em></td>
<td>Antimalarial, fever, diarrheahoea, cardiovascular disease, and antioxidant</td>
<td>7.2 µg/mL (MeOH)</td>
<td>Increased levels of inactive p-GSK3β in Alzheimer’s disease</td>
<td>Mishra et al., 2011; Gu et al., 2020</td>
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</table>
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 chemosuppression of 60.7% and 36.1% (p<0.05) respectively. Furthermore, animals infected with NK65 and ANKA strains administered with quercetin respectively displayed increase in pGSK3β (Ser9) at 2.3-fold and 1.2-fold respectively. In addition to that, quercetin also modulated cytokine 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 anti-inflammatory 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 antiplasmodial cytokine response also showed that P. berghei-infected animals proinflammatory cytokines (TNF-α and IFN-γ) were lowered and anti-inflammatory 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 antiplasmodial activity via 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 significant 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 (IC50 = 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 P. tularensis infections (Zhang et al., 2009) reduced proinflammatory cytokine production whilst elevating production of antiinflammatory cytokines. Findings from 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 GSK3β.

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.](image-url)
<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>Properties</th>
<th>In vitro antiplasmodial activity against <em>P. falciparum</em> 3D7 (IC₅₀)</th>
<th>In vivo antimalarial effects against <em>P. berghei</em> NK65 (chemo-suppression percentage)</th>
<th>GSK3 inhibition properties (Western blotting analysis)</th>
<th>Cytokine modulating effect</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Kaempferol</td>
<td>Antimalarial, antiinflammation, antidiabetic, antioxidant, cardioprotective effect, reduced risk of pancreatic cancer</td>
<td>30.94 ± 1.48 μM</td>
<td>60.27 ± 3.20 % (5 mg/kg; therapeutic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)</td>
<td>Wong et al., 2015</td>
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<td>60.33 ± 4.50 % (20 mg/kg; prophylactic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (prophylactic treatment)</td>
<td>Hassan, 2019b</td>
<td></td>
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<tr>
<td>Methyl-4-hydroxycinnamate</td>
<td>Antimalarial, antiinflammation, antifungal</td>
<td>8.41 ± 1.25 μM</td>
<td>68.44 ± 8.29 % (30 mg/kg; therapeutic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)</td>
<td>Sudi et al., 2018; Hassan, 2019b</td>
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<td>78.35 ± 8.13 % (60 mg/kg; prophylactic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (prophylactic treatment)</td>
<td>Hassan, 2019b</td>
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<tr>
<td>Curcumin</td>
<td>Antimalarial, antiinflammation, antidiabetic, fever, skin disease, antibacterial, antiviral</td>
<td>4.34 ± 1.59 μM</td>
<td>67.61 ± 1.71 % (30 mg/kg; therapeutic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)</td>
<td>Ali et al., 2017</td>
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<td>57.40 ± 1.33 % (30 mg/kg; prophylactic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (prophylactic treatment)</td>
<td>Ali et al., 2017</td>
<td></td>
</tr>
<tr>
<td>Andrographolide</td>
<td>Antimalarial, antiinflammation, antifungal</td>
<td>13.7 ± 0.86 μM</td>
<td>60.17 ± 2.12 % (5 mg/kg; therapeutic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)</td>
<td>Hassan et al., 2019a</td>
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<td>60.82 ± 6.69 % (15 mg/kg; prophylactic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)</td>
<td>Hassan et al., 2019a</td>
<td></td>
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<tr>
<td>Quercetin</td>
<td>Antimalarial, antiinflammation</td>
<td>19.31 ± 1.26 μM</td>
<td>67.61 ± 1.71 % (30 mg/kg; therapeutic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)</td>
<td>Ali et al., 2021; Hassan, 2019b</td>
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<td>57.40 ± 1.33 % (30 mg/kg; prophylactic treatment)</td>
<td>↑IL-10; ↑IL-4; ↓TNF-α; ↓IFN-γ (therapeutic)</td>
<td>Ali et al., 2021; Hassan, 2019b</td>
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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-hydroxyxinnamate, 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 inflammatory 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) (Khán 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.
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, 7-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 |
| DvI/2/3 | Dishvelled 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-ζ | tumor necrosis factor alpha |
| Wnt | Wingless-integrated gene site member |

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