Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Antimalarial potential of Gymnema inodorum leaf extract and dihydroartemisinin combination against Plasmodium berghei infected mice

Sakaewan Ounjaijean
Chiang Mai University
Voravuth Somsak (voravuth.so@wu.ac.th)
Walailak University

Article

Keywords: Antimalarial potential, Gymnema inodorum, Plasmodium berghei, Dihydroartemisinin, Combination

Posted Date: May 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1520692/v2

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Chemotherapy plays a crucial role in malaria control. However, the main obstacle to treatment has been the rise of parasite resistance to most antimalarial drugs. Artemisinin-based combination therapies (ACTs) remain today's most effective antimalarial therapies. However, malaria parasite tolerance to ACTs is now increasingly prevalent, especially in Southeast Asia. Consequently, this creates the need for effective alternative antimalarials. Interestingly, the effectiveness of *Gymnema inodorum* leaf extract (GIE) as an antimalarial candidate was reported earlier. Therefore, this study sought to evaluate the antimalarial potential of dihydroartemisinin (DHA) based combination therapy with GIE against *Plasmodium berghei* in a mouse model. Drug assessment was carried out on *P. berghei* ANKA (PbANKA) using the standard 4-day test for determination of fifty percent effective dose (ED_{50}) of DHA and GIE individually. Moreover, the combination of DHA and GIE was also evaluated based on fixed-ratio strategy, 100/0, 80/20, 60/40, 40/60, 20/80, and 0/100 of ED_{50} of DHA and GIE, respectively. Overall, 2 mg/kg and 100 mg/kg of DHA and GIE resulted in ED_{50} against PbANKA. In combination, the ratio of 60/40 (DHA/GIE) had a considerable the highest antimalarial activity significantly ($p < 0.001$) against PbANKA with 88.95% inhibition that indicated a synergism efficacy (CI value = 0.68695). Additionally, this ratio was protected PbANKA infected mice from BW loss and PCV reduction with prolonged survival time throughout the 30 day follow up. This study points to better efficacy of GIE as an alternative drug partner in combination to enhance antimalarial efficacy of DHA against PbANKA infected mice.

Introduction

Malaria is a parasitic disease that remains one of the significant problems and affects mostly the population living in the developing countries in Africa, Asia, Latin America, and South-West Pacific. Malaria in humans is caused by Apicomplexan protozoan parasites of the genus *Plasmodium*, including *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, and transmitted by the female *Anopheles* mosquito (White et al. 2014). There were approximately 1.5 billion malaria cases averted since 2000, and 229 million new malaria cases with 409,000 deaths worldwide in 2019, especially pregnant women and children under five years (WHO 2019). With challenges in vector control and the development of effective vaccine, chemotherapy remains the mainstay of the malaria control strategy. However, the emergence and spread of malaria parasites resistant to many of the available antimalarial drugs today is, therefore, a major cause for concern (Tang et al. 2020). As a result, artemisinin-based combination therapy (ACT) was adopted and is currently recommended by the World Health Organization (WHO) as the first-line regimen for the treatment of uncomplicated malaria (Sinclair et al. 2009). Unfortunately, artemisinin resistance and reduction of ACT efficacy by malaria parasites cases of resistance in the Thailand-Cambodia border, Southeast Asia have been noted (Koehne et al. 2021). Hence, there is still an urgent need to discover and develop a new effective drug combination of treating malaria, including medicinal plants as traditional medicine. Traditional medicine has been used for a long history in treating various diseases, including malaria. It is currently well known that many secondary metabolites derived from medicinal plants play an essential role in a wide range of therapeutic effects. The WHO recognizes the

vital role of traditional medicines and continues to support the integration of conventional medicine into each country's health system (Degotte et al. 2021).

Gymnema inodorum (Lour.) Decne is the plant belonging to the family Asclepiadaceae, and found ubiquitously in Southeastern Asia and Thailand, especially Northern region. It is widely used as local Thai vegetables and commercial herb tea products. It has been known to have therapeutic effects as mentioned in folk medicine, Ayurveda, and homeopathic systems of medicine. Traditionally, it has been used in curing certain diseases, including diabetes mellitus, rheumatic arthritis, and gout (Dunkhunthod et al. 2021). The leaves of *G. inodorum* were investigated to have many therapeutic phytochemical compounds such as phenolics, flavonoids, terpenoids, triterpenoid saponin, and glycoside. These compounds have been reported to have multiple therapeutic potentials, including antioxidant, anti-inflammation, anti-diabetic and hypoglycemic, anti-adipogenesis, anti-microbial, and anti-cancer activities (Kahksha et al. 2022). Previous studies of *G. inodorum* leaf extract using in vivo model showed that this plant presented potent antimalarial activity and protection of hypoglycemia, dyslipidemia, liver damage, and acute kidney injury with normalization of hematological parameters against *Plasmodium berghei* infection in mice (Boonyapranai et al. 2021; Ounjaijean et al. 2021a; Ounjaijean et al. 2021b). However, to the best of our knowledge, this plant's antimalarial activity in combination treatment has not yet been reported, especially for the research study in *P. berghei*. Therefore, this study was the first report investigating the antimalarial potential of interaction between *G. inodorum* leaf extract and dihydroartemisinin against *P. berghei* infection in experimental mice.

Materials And Methods

Gymnema inodorum and preparation of extract

Gymnema inodorum leaves were obtained from the Chiangda Organic Company Garden, Chiang Mai, Thailand. A plant biologist authenticated this plant at Chiang Mai University and the voucher specimen (NRU64/036-001) was subsequently deposited at the Research Excellence Center for Innovation and Health Products, Walailak University. The plant materials were dried in a hot-air oven at 50^{o}C, and dried powdered material was then prepared using the electric blender. To prepare of aqueous crude extract, 250 g of the dried powdered *G. inodorum* was soaked in 750 ml of distilled water at room temperature for seven days with occasional stirring. Filtration was then performed with Whatman no. 1 filter paper and collect the filtrate. Lyophilization was carried out to obtain the dried powdered form of aqueous crude extract of *G. inodorum* (GIE) and stored at –20^{o}C until further use (Ounjaijean et al. 2021a). Before experiments, GIE at the chosen doses was freshly prepared in 20% Tween-80 according to the weight of the mice.

Preparation of standard antimalarial drug

Dihydroartemisinin (DHA) was obtained from Sigma-Aldrich Co. (St Louis, MO, U.S.A.) and stored at -20^{o}C. For experiments, DHA at the chosen doses was dissolved in 20% Tween-80 according to the mice'

body weight for administering orally.

Experimental animals

Healthy Balb/c male mice, 4-6 weeks old, weighing 20-25 g at the time of primary infection obtained from Nomura Siam International Co., Ltd., were used throughout the study. The mice were kept in a room with temperature control between 22-25^{o}C and 12 h light/12 h dark cycle. They were fed on a commercial pellet diet 082G and clean tap water *ad libitum*.

Rodent malaria parasite

Plasmodium berghei strain ANKA (PbANKA) obtained from MR4 (Malaria Research and Reference Reagent Resource Center, https://www.beiresources.org/About/MR4.aspx) was used in this study. Cryopreservative stock of PbANKA was thawed in a water bath at 37^{o}C, and 200 ml of the suspension was inoculated by intraperitoneal (IP) injection into naïve Balb/c mice. Parasitemia was monitored daily by microscopic examination of Giemsa-stained blood film, and serial passage was then performed when parasitemia reached about 10-20%. Blood was collected by cardiac puncture and diluted with normal saline to obtain 1x10^{7} parasitized erythrocytes for IP injection.

Determination of parasitemia

Tail blood from PbANKA infected mice was smeared on a microscopic slide. After air-dried, smeared slide was then fixed with absolute methanol and stained with 10% Giemsa solution for 15 min at room temperature. Parasitized erythrocytes were counted under a light microscope with a 100x oil immersion lens, and parasitemia was subsequently calculated using the following formula.

$$\% \text{ parasitemia} = \frac{\text{Number of parasitized erythrocytes x 100}}{\text{Total number of erythrocytes}}$$

Antimalarial assay

The antimalarial assay was first carried out to determine the effective dose (ED_{50}) of the individual substances (GIE and DHA) according to standard 4-day suppressive test as previously described (Peters 1975). Groups of mice (5 mice/group) were inoculated with 1x10^{7} parasitized erythrocytes of PbANKA by IP injection. Two hours after infection, mice were administered orally by gavage with GIE (1, 10, 50, 100, and 200 mg/kg) and DHA (0.1, 1, 5, 10, 20 mg/kg) once a day for 4-consecutive days (day 0-3). The untreated control was given 10 ml/kg of 2% Tween-80. On day 4, parasitemia was determined by microscopic examination of Giemsa-stained blood film, and the percentage of inhibition was subsequently calculated using the following formula.

$$\% \text{ inhibition} = \frac{(\text{Parasitemia of untreated group} - \text{Parasitemia of tested group}) \times 100}{\text{Parasitemia of untreated group}}$$

Combination treatment

The obtained ED_{50} values of both GIE and DHA were used for combination treatment. The combination was prepared in fixed ratios of 100/0, 80/20, 60/40, 40/60, 20/80, and 0/100 of GIE/DHA according to the fixed ratio method as previously described (Nateghpour et al. 2012). The standard 4-day suppressive test was used to test the combination treatment in this step. On day 4, parasitemia was estimated, and % inhibition was then calculated. Interaction between DHA and GIE against the PbANKA was interpreted as lying the points above the joint line indicated synergism and either around the line or below indicated additive or antagonism, respectively. A combination index was also calculated to interpret the interaction between GIE and DHA against PbANKA. Moreover, body weight, packed cell volume, and mean survival time for each group were recorded.

Determination of body weight and packed cell volume

Each mouse's body weight (BW) in all groups was measured and recorded using sensitive electronic balance before infection on day 0 and on day 4 post-infection. To determine packed cell volume (PCV), blood was collected from the tail vein of each mouse in heparinized capillary tubes by filling ¾ of its volume. The tubes were sealed and centrifuged at 12,000 rpm for 15 min using a microhematocrit centrifuge. PCV was then calculated using the following formula before infection on day 0 and day 4 post-infection.

$$\text{PCV} = \frac{\text{Volume of packed erythrocytes} \times 100}{\text{Total blood volume}}$$

Mean survival time

The mortality of each mouse was monitored and recorded from the time of infection until death throughout the follow-up period (30 days). Mean survival time (MST) was determined using the following formula.

$$\text{MST} = \frac{\text{Sum of survival time of mice in a group}}{\text{Total number of mice in that group}}$$

Statistical analysis

GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA) was used to analyze the results of this study. Data were presented as the mean ± standard error of the mean (SEM). The non-linear regression function for the sigmoidal dose-response variable slope was conducted to obtain the best-fit ED_{50} value. The comparison between the mean of control and treatment groups was tested with one-way analysis of variance (ANOVA) and Tukey's post-test. The 95% confidence, $p < 0.05$ was considered as statistical significances. Moreover, combination index (CI) was automatically simulated by CompuSyn software (ComboSyn, Inc., USA), which defines synergism (CI < 1), additive effect (CI = 1), and antagonism (CI > 1).

Results

PbANKA development in Balb/c mice

In order to investigate the development of PbANKA in mice, parasitemia, BW, PCV, and MST were monitored. As shown in Fig. 1A, parasitemia was first detectable on day 1 post-infected with parasitemia lower than 1% and reached 51% on day 10 post-infection. Next, we observed that BW and PCV were markedly decreased in infected Balb/c mice (Fig. 1B and 1C). We also observed that PbANKA infected mice died within 10 days post-infection (Fig. 1D).

Antimalarial activities of DHA and GIE

In order to investigate the antimalarial activity of DHA and GIE against PbANKA, the standard 4-day suppressive test was carried out. Figure 2A showed that GIE significantly ($p < 0.05$) inhibited PbANKA in a dose-dependent manner at 50, 100, and 200 mg/kg doses with 32%, 50%, and 65% inhibition, respectively. However, 1 and 10 mg/kg of GIE did not show an antimalarial effect. Moreover, significant ($p < 0.01$) antimalarial activity with a dose-dependent manner was also observed in the infected mice treated with DHA at the doses of 1, 5, 10, and 20 mg/kg with 32%, 60%, 95%, and 100%, respectively. The calculated ED_{50} values of DHA and GIE against PbANKA were obtained 2.06 (~ 2) and 101.2 (~ 100) mg/kg, respectively (Fig. 2B).

Interaction of DHA and GIE against PbANKA infected mice

In order to investigate the effect of combination treatment against PbANKA, the interaction of DHA and GIE in various combinations was carried out. The results of antimalarial activity in combination treatment were shown in Fig. 3 and Table 1. The ratio of 60/40 (DHA/GIE) had a significant potentiation against PbANKA. This ratio had the highest antimalarial activity significantly ($p < 0.001$) with 88.95% inhibition, compared to untreated control that indicated a synergism efficacy (CI value = 0.68695). The curve obtained by other combinations of DHA and GIE and CI values were evidence of an antagonistic interaction.

Table 1
Combination index of interaction between DHA and GIE against PbANKA infected mice

Test		Dose (mg/kg		CI value
		DHA	**GIE**	
Combination	100/0	2	0	-
	80/20	1.6	20	1.70625[b]
	60/40	1.2	40	0.68695[a]
	40/60	0.8	60	1.48699[b]
	20/80	0.4	80	3.65127[b]
	0/100	0	100	-

[a] CI < 1; synergism, [b] CI > 1; antagonism

Effects of interaction of DHA and GIE on BW, PCV, and MST in PbANKA infected mice

In order to investigate the effects of combination treatment between DHA and GIE on complications during PbANKA infection, the BW, PCV, and MST were measured. PbANKA infection significantly ($p < 0.01$) decreased BW and PCV, compared to healthy control (Fig. 4A and 4B). As expected, the infected mice treated with the ratio of 60/40 between DHA and GIE were protected from BW loss and PCV reduction with significance ($p < 0.05$), compared to untreated and DHA treated alone. Interestingly, the ratio of 40/60, 20/80, and 0/100 that increased the proportion of GIE also showed significant ($p < 0.05$) protective effects on BW loss and PCV reduction. Additionally, the MST of combination treatment between DHA and GIE at the ratio of 60/40 was significantly ($p < 0.05$) longer than other groups throughout the 30 day follow-up (Fig. 4C). Moreover, the 40/60, 20/80, and 0/100 between DHA and GIE also showed significantly ($p < 0.05$) prolonged MST, compared to untreated and DHA treated alone.

Discussion

With the emergence of antimalarial drug resistance, the search for new treatment options is urgently needed. New drugs are also needed to be used as com-bination with the standard antimalarial drugs, particularly artemisinin-based combination (Sinclair et al. 2009). In the present study, the antimalarial activity of GIE, an indigenous medicinal plant, and functional food in Thailand, in combination with DHA on mice infected with PbANKA was reported. The mice treated with 50, 100, and 200 mg/kg of GIE showed significant 32%, 50%, and 65% inhibition, respectively, compared to untreated control. It has been described that *in vivo* antimalarial activity of plant extract in which > 30% inhibition makes this extract to be considered active (Krettli et al. 2009). Hence, GIE can be classified as active antimalarial activity and in agreement with the previous report (Ounjaijean et al. 2021a). This can be explained by the fact that

antimalarial activity of GIE could have resulted from single or in the combined action of the bioactive metabolites such as phenols, flavones, alkaloids, anthraquinones, quinones, tannin, and triterpene saponins (Dunkhunthod et al. 2021; Khan et al. 2019; Rasoanaivo et al. 2011). Gymnemic acids, major active compounds of GIE, might play a vital role in exerting antimalarial activity (Kanetkar et al. 2007; Sahu et al. 1996; Saiki et al. 2020). The possible antimalarial mechanisms might be through antioxidant, intercalate with parasite DNA, inhibiting the fatty acid and protein biosynthesis of the parasite, elevating erythrocyte oxidation, immunomodulatory, inhibition of parasite invasion, or by other unknown mechanisms (Fidock et al. 2004; Trang et al. 2021).

We also tested GIE in combination with DHA on mice infected with PbANKA. The combination treatment ratio of 60/40 (DHA/GIE) was more effective than the others with 88.95% inhibition, and synergism was observed in this ratio. Significant anti-malarial activity of GIE in combination with DHA was found when compared to either GIE or DHA monotherapies. It is noteworthy that DHA has been proposed several mechanisms of action, including the production of free radicals or reactive metabolites and inhibition of nutrient flow to the parasite by interfering membrane transport properties (Dai et al. 2021; Guo 2016; Tilley et al. 2016). Moreover, DHA is an inhibitor of SERCA (SE sarco/endoplasmic reticulum Ca2+ - ATPase) of parasite and is a suitable target for artemisinin and its derivatives (Lu et al. 2015). GIE might be associated with different metabolic pathways, and as expected, the combination of GIE and DHA showed the best synergy with beneficial results. However, more work is needed before any firm conclusion.

Loss of BW is one manifestation of malaria infection in mice, and BW change of mice is a parameter for evaluating the antimalarial activity of extract (Basir et al. 2012). PbANKA infected mice treated with DHA/GIE combination at the doses of ratios 60/40, 40/60, 20/80, and 0/100 showed a significant increase in BW compared to the untreated control and DHA monotherapy suggested the effect of GIE in preventing malaria-related BW loss. This could be attributed to the presence of compounds in GIE that affect appetite leading to protect BW loss during malaria infection (Pinent et al. 2017). However, the activity of GIE in the combination ratio of 80/20 was not strong enough to significantly prevent BW loss.

Additionally, reduction of PCV during malaria infection is also considered. The PCV of untreated control was reduced because of PbANKA infection leading to rapid hemolysis (Khobjai et al. 2014; Zhu et al. 2015). The absence of significant PCV reduction among DHA/GIE combination-treated mice at the doses of ratios 60/40, 40/60, 20/80, and 0/100 may indicate the protective effect of GIE on PCV reduction during malaria infection. This could be due to activating erythropoietin and sustaining the availability of new erythrocytes production in the bone marrow. This finding agrees with the previous studies that showed the protective effect of GIE on PCV reduction in rodent malaria (Ounjaijean et al. 2021b).

MST is another parameter to evaluate the antimalarial activity of plant extracts. In this study, all doses of DHA/GIE combination significantly prolonged MST compared to untreated control, especially at the ratio of 60/40. These further supplements the evidence on inhibition of PbANKA resulting in a reduced overall parasite pathology on the experimental mice. A plant extract that can prolong MST of infected mice

compared to untreated control is considered active (Oliveira et al. 2009). Hence, GIE, in combination with DHA, is regarded as active for antimalarial activity against PbANKA.

All results in this study provided the first evidence-based antimalarial activity with the synergistic effect of GIE in combination with DHA against PbANKA infected mice. Moreover, the combination between DHA and GIE also showed protective effects on BW loss and PCV reduction induced by malaria infection with prolonged MST. This study recommends GIE as an alternative antimalarial substance for using with standard antimalarial drugs such as DHA in the future. However, modes of action and possible mechanisms of GIE and its combination with DHA in malarial treatment should be further investigated.

Declarations

Acknowledgements

The authors were grateful to the staff of Laboratory Animal Unit, Research Institute for Health Sciences, Walailak University for their tremendous technical assistance and animal operations. Special thanks were due to Prof. Dr. Somdet Srichairatanakool and Dr. Chairat Uthaipibull for their helpful suggestion.

Funding

This research work was partially supported by Chiang Mai University, Chiang Mai, Thailand.

Conflicts of interest / Competing interests

The authors declare that they have no conflicts of interest.

Availability of data and material

The data used to support the finding of this study are available from the corresponding author upon request.

Authors' contributions

Conceptualization, methodology, validation, formal analysis, writing-review and editing, supervision, and project administration were done by V.S. Data curation and writing-original draft preparation were done by S.O. All authors have read and agreed to the published version of the manuscript.

Ethics approval

All experiments involving animals were conducted under the NIH Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee, Walailak University (WU-ACUC-65002).

Consent to participate

Not applicable

Consent for publication

Authors are responsible for correctness of the statements provided in the manuscript.

References

1. Basir R, et al. (2012) Plasmodium berghei ANKA Infection in ICR Mice as a Model of Cerebral Malaria. Iran J Parasitol 7(4):62–74
2. Boonyapranai K, Surinkaew S, Somsak V, Rattanatham R (2021) Protective Effects of *Gymnema inodorum* Leaf Extract on *Plasmodium berghei*-Induced Hypoglycemia, Dyslipidemia, Liver Damage, and Acute Kidney Injury in Experimental Mice. J Parasitol Res 2021:1896997 doi:10.1155/2021/1896997
3. Dai X, et al. (2021) Dihydroartemisinin: A Potential Natural Anticancer Drug. Int J Biol Sci 17(2):603–622 doi:10.7150/ijbs.50364
4. Degotte G, Pirotte B, Francotte P, Frederich M (2021) Overview of Natural Antiplasmodials from the Last Decade to Inspire Medicinal Chemistry. Curr Med Chem 28(30):6199–6233 doi:10.2174/0929867328666210329112354
5. Dunkhunthod B, Talabnin C, Murphy M, Thumanu K, Sittisart P, Eumkeb G (2021) *Gymnema inodorum* (Lour.) Decne. Extract Alleviates Oxidative Stress and Inflammatory Mediators Produced by RAW264.7 Macrophages. Oxid Med Cell Longev 2021:8658314 doi:10.1155/2021/8658314
6. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S (2004) Antimalarial drug discovery: efficacy models for compound screening. Nat Rev Drug Discov 3(6):509–20 doi:10.1038/nrd1416
7. Guo Z (2016) Artemisinin anti-malarial drugs in China. Acta Pharm Sin B 6(2):115–24 doi:10.1016/j.apsb.2016.01.008
8. Kahksha, et al. (2022) Recent developments made in the assessment of the antidiabetic potential of gymnema species - From 2016 to 2020. J Ethnopharmacol 286:114908 doi:10.1016/j.jep.2021.114908
9. Kanetkar P, Singhal R, Kamat M (2007) *Gymnema sylvestre*: A Memoir. J Clin Biochem Nutr 41(2):77–81 doi:10.3164/jcbn.2007010
10. Khan F, et al. (2019) Comprehensive Review on Phytochemicals, Pharmacological and Clinical Potentials of *Gymnema sylvestre*. Front Pharmacol 10:1223 doi:10.3389/fphar.2019.01223
11. Khobjai W, Jaihan U, Watcharasamphankul W, Somsak V (2014) Protective effect of *Thunbergia laurifolia* extract on hemolysis during *Plasmodium berghei* infection. Parasitol Res 113(5):1843–6 doi:10.1007/s00436-014-3831-y
12. Koehne E, Adegnika AA, Held J, Kreidenweiss A (2021) Pharmacotherapy for artemisinin-resistant malaria. Expert Opin Pharmacother 22(18):2483–2493 doi:10.1080/14656566.2021.1959913

13. Krettli AU, Adebayo JO, Krettli LG (2009) Testing of natural products and synthetic molecules aiming at new antimalarials. Curr Drug Targets 10(3):261–70 doi:10.2174/138945009787581203
14. Lu M, Sun L, Zhou J, Zhao Y, Deng X (2015) Dihydroartemisinin-Induced Apoptosis is Associated with Inhibition of Sarco/Endoplasmic Reticulum Calcium ATPase Activity in Colorectal Cancer. Cell Biochem Biophys 73(1):137–45 doi:10.1007/s12013-015-0643-3
15. Nateghpour M, Farivar L, Souri E, Hajjaran H, Mohebali M, Motevalli Haghi A (2012) The Effect of *Otostegia persica* in Combination with Chloroquine on Chloroquine-Sensitive and Chloroquine-Resistant Strains of *Plasmodium berghei* Using *In-vivo* Fixed Ratios Method. Iran J Pharm Res 11(2):583-8
16. Oliveira AB, Dolabela MF, Braga FC, Jacome RL, Varotti FP, Povoa MM (2009) Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids. An Acad Bras Cienc 81(4):715–40 doi:10.1590/s0001-37652009000400011
17. Ounjaijean S, Romyasamit C, Somsak V (2021a) Evaluation of Antimalarial Potential of Aqueous Crude *Gymnema Inodorum* Leaf Extract against *Plasmodium berghei* Infection in Mice. Evid Based Complement Alternat Med 2021:9932891 doi:10.1155/2021/9932891
18. Ounjaijean S, Sukati S, Somsak V, Sarakul O (2021b) The Potential Role of *Gymnema inodorum* Leaf Extract Treatment in Hematological Parameters in Mice Infected with *Plasmodium berghei*. J Trop Med 2021:9989862 doi:10.1155/2021/9989862
19. Peters W (1975) The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of *Plasmodium berghei* in screening for blood schizontocidal activity. Ann Trop Med Parasitol 69(2):155–71
20. Pinent M, Blay M, Serrano J, Ardevol A (2017) Effects of flavanols on the enteroendocrine system: Repercussions on food intake. Crit Rev Food Sci Nutr 57(2):326–334 doi:10.1080/10408398.2013.871221
21. Rasoanaivo P, Wright CW, Willcox ML, Gilbert B (2011) Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. Malar J 10 Suppl 1:S4 doi:10.1186/1475-2875-10-S1-S4
22. Sahu NP, Mahato SB, Sarkar SK, Poddar G (1996) Triterpenoid saponins from *Gymnema sylvestre*. Phytochemistry 41(4):1181–5 doi:10.1016/0031-9422(95)00782-2
23. Saiki P, et al. (2020) Purified Gymnemic Acids from *Gymnema inodorum* Tea Inhibit 3T3-L1 Cell Differentiation into Adipocytes. Nutrients 12(9) doi:10.3390/nu12092851
24. Sinclair D, Zani B, Donegan S, Olliaro P, Garner P (2009) Artemisinin-based combination therapy for treating uncomplicated malaria. Cochrane Database Syst Rev(3):CD007483 doi:10.1002/14651858.CD007483.pub2
25. Tang YQ, Ye Q, Huang H, Zheng WY (2020) An Overview of Available Antimalarials: Discovery, Mode of Action and Drug Resistance. Curr Mol Med 20(8):583–592 doi:10.2174/1566524020666200207123253

26. Tilley L, Straimer J, Gnadig NF, Ralph SA, Fidock DA (2016) Artemisinin Action and Resistance in *Plasmodium falciparum*. Trends Parasitol 32(9):682–696 doi:10.1016/j.pt.2016.05.010
27. Trang DT, et al. (2021) Pregnane glycosides from *Gymnema inodorum* and their alpha-glucosidase inhibitory activity. Nat Prod Res 35(13):2157–2163 doi:10.1080/14786419.2019.1663517
28. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM (2014) Malaria. Lancet 383(9918):723–35 doi:10.1016/S0140-6736(13)60024-0
29. WHO (2019) World Health Organization, World Malaria Report. http://wwwwhoint/malaria/world_malaria_report_2019/en/indexhtml
30. Zhu X, et al. (2015) Phenylhydrazine administration accelerates the development of experimental cerebral malaria. Exp Parasitol 156:1–11 doi:10.1016/j.exppara.2015.05.011

Figures

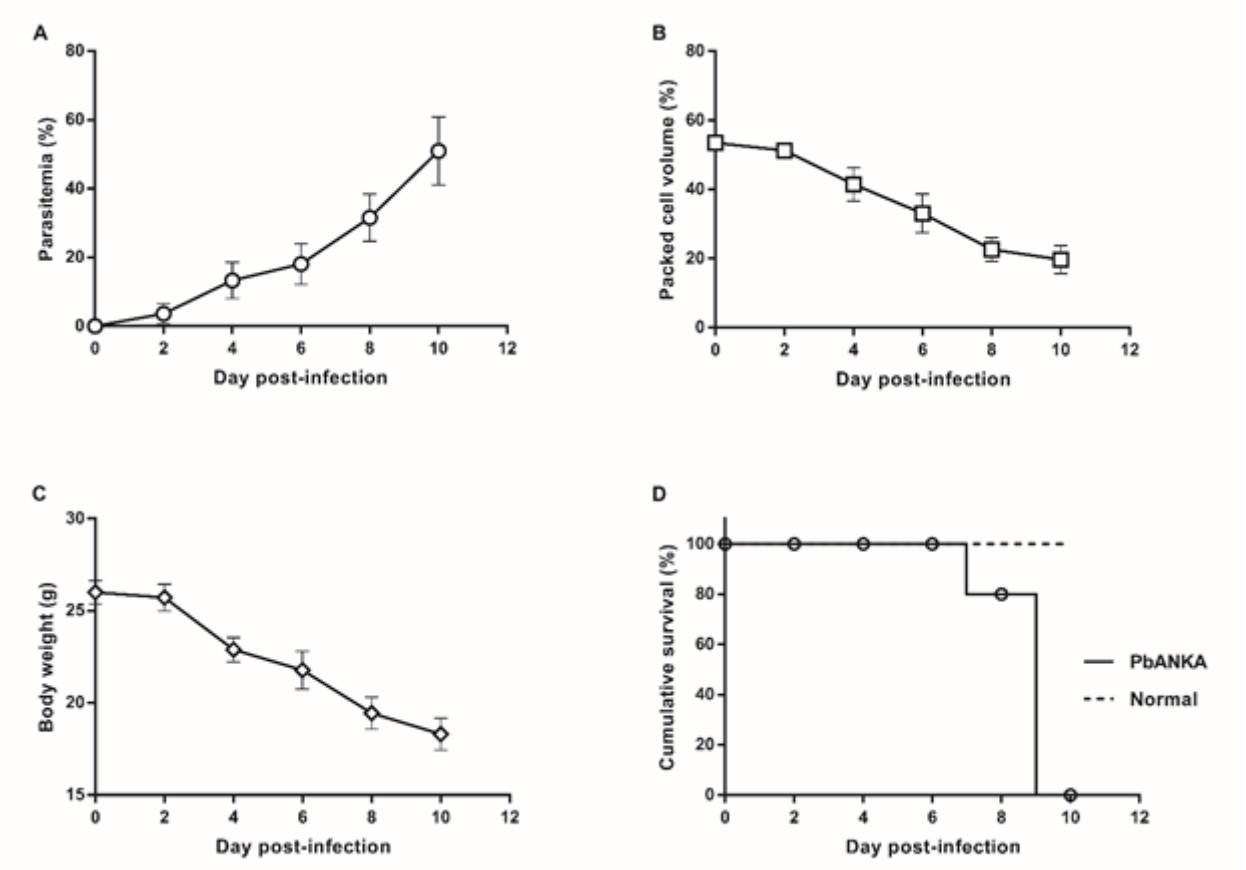


Figure 1

PbANKA infection in mice. Balb/c mice were infected with $1x10^7$ parasitized erythrocytes of PbANKA by IP injection. (A) Parasitemia, (B) BW, (C) PCV, and (D) cumulative survival were monitored. Results were presented as mean ± SEM (n = 5).

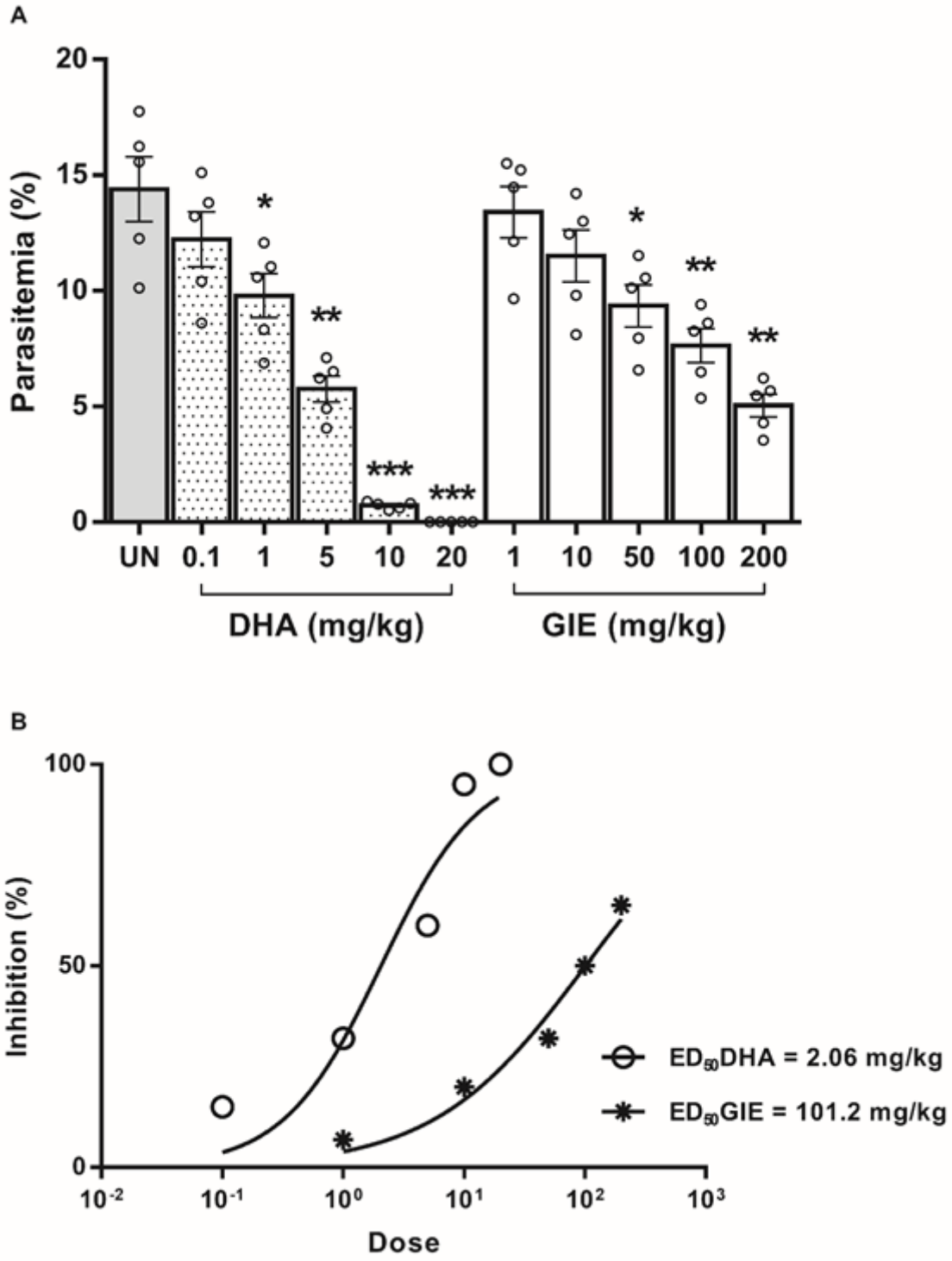


Figure 2

Antimalarial activity of DHA and GIE with their ED_{50} values against PbANKA infected mice. Balb/c mice were inoculated with $1x10^7$ parasitized erythrocytes of PbANKA by IP injection. They were subsequently administered orally by gavage with DHA (0.1, 1, 5, 10, and 20 mg/kg) and GIE (1, 10, 50, 100, and 200 mg/kg) for 4-consecutive days. On day 4, (A) parasitemia and (B) ED_{50} values were calculated. The

results were presented as mean ± SEM (n = 5). UN; untreated control, DHA; dihydroartemisinin, GIE; *Gymnema inodorum* leaf extract. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, compared to untreated control.

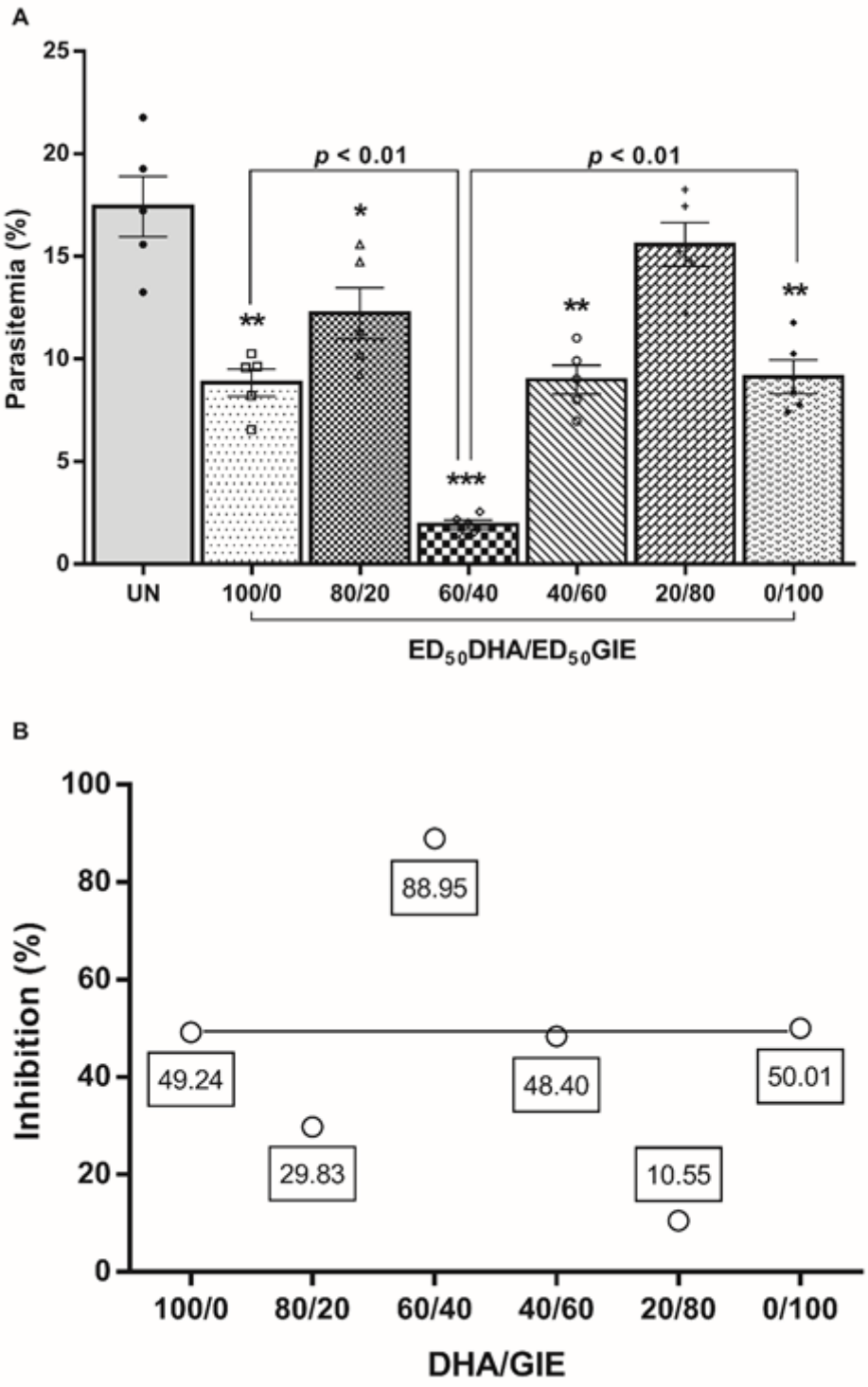


Figure 3

Interaction of DHA and GIE against PbANKA infected mice. Balb/c mice were inoculated with $1x10^7$ parasitized erythrocytes of PbANKA by IP injection. They were given with combination between the ED_{50}

value of DHA and ED_{50} value of GIE (100/0, 80/20, 60/40, 40/60, 20/80, and 0/100) by oral gavage for 4-consecutive days. On day 4, (A) parasitemia was measured, and (B) interaction line was generated. The results were presented as mean ± SEM (n = 5). UN; untreated control, DHA; dihydroartemisinin, GIE; *Gymnema inodorum* leaf extract. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, compared to untreated control.

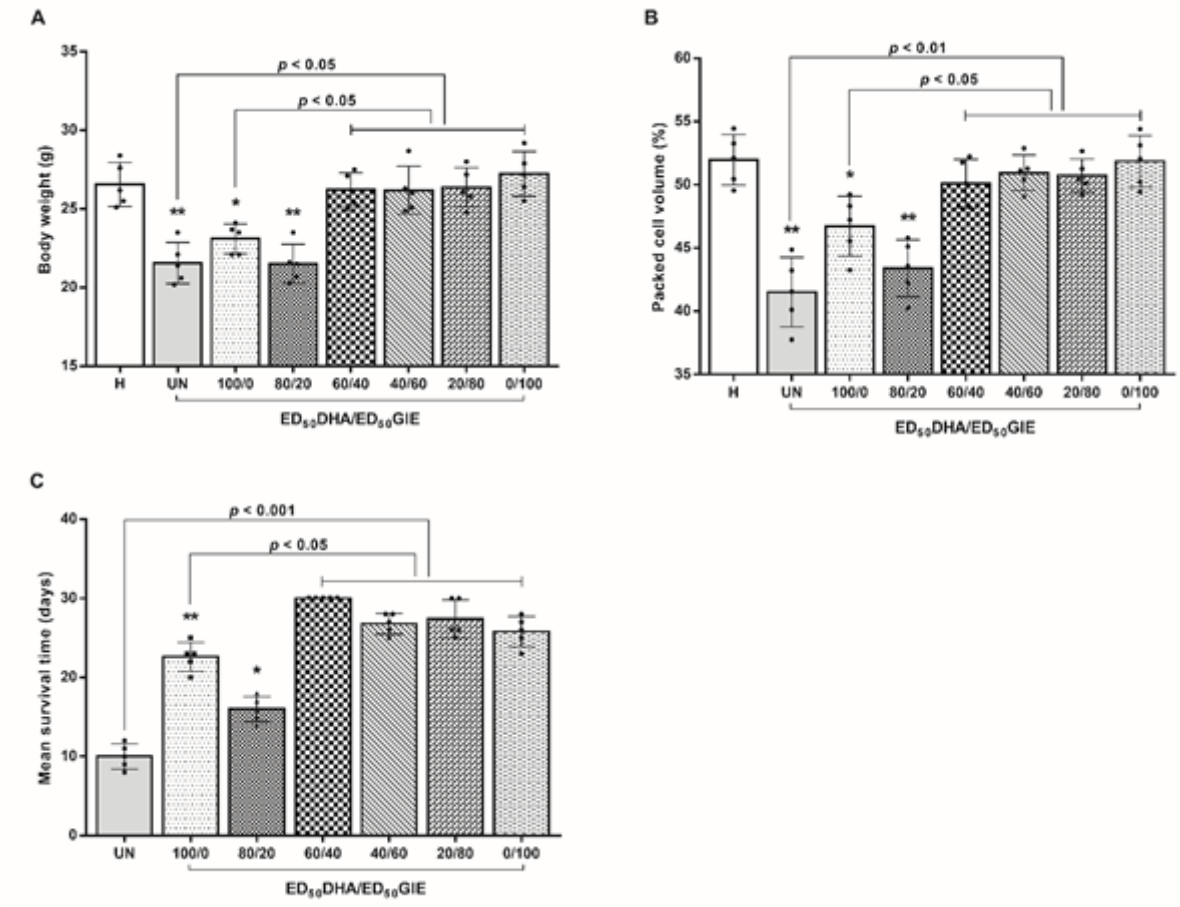


Figure 4

BW, PCV, and MST of PbANKA infected mice treated with the interaction of DHA and GIE. Balb/c mice were inoculated with $1x10^7$ parasitized erythrocytes of PbANKA by IP injection. They were given with combination between the ED_{50} value of DHA and ED_{50} value of GIE (100/0, 80/20, 60/40, 40/60, 20/80, and 0/100) by oral gavage for 4-consecutive days. On day 4, (A) BW, (B) PCV, and (C) MST were measured. The results were presented as mean ± SEM (n = 5). H; healthy control, UN; untreated control, DHA; dihydroartemisinin, GIE; *Gymnema inodorum* leaf extract. * $p < 0.05$ and ** $p < 0.01$, compared to healthy control.