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Towards Andrographolide as Antimalarial: A Systematic Review

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Antimalarial resistance remains a problem in malaria elimination programs worldwide. Andrographolide is a labdane diterpenoid extracted form Andrographis paniculata that introduced as a new antimalarial agent, but its potential to prevent resistance must be elaborated to mitigated risk of resistance. This systematic review aimed to analyze the potential of andrographolide to prevent plasmodium resistance. A systematic review was made by comprehensive searching in several database such as PubMed, Google scholar, Science direct with keyword: andrographolide, malaria, plasmodium, resistance, review with several method of study includes in vivo, in vitro, in silico and randomized control trial. We mapped research development of andrographolide to be antimalarial agent using Vos viewer to extract co-occurrence terms that related to andrographolide as antimalarial. Based on PRISMA results, 35 articles meet criteria. Type of andrographolide and its derivative use as treatment, type of study, control, analytical method and result of mechanism of action was compiled within the review table. Andrographolide effective as antimalarial agent, its mechanism includes suppressing parasite numbers, suppressing the inflammatory response in malaria, prevent detoxifying heme, immunomodulating host cells, potent antioxidant, and inhibiting enzymes that play a role in parasite invasion. Further research of andrographolide to prevent antimalarial resistance in Plasmodium must investigated deeply.

Keywords: Andrographolide; Malaria; Plasmodium; Resistance.

*A*ndrographolide ($C_{20}H_{30}O_5$), an active component of the *Andrographis paniculata* (Burm.f.) Wall. ex Nees , has been distributed and utilized for centuries in traditional medical systems throughout Asia.[1] Andrographolide's antimalarial characteristics were well-known in the late twentieth century.[2,3] *Andrographis* has traditionally been used to cure a range of diseases, including fevers that are symptoms of malaria. Initial studies focused on its effects against malaria parasites, primarily in vitro.[4] More recent research has confirmed the antimalarial activity of

andrographolide through various in vitro assays. Studies have shown that it has inhibitory effects on *Plasmodium falciparum*. Animal studies have also supported the potential of andrographolide as an antimalarial agent. These studies often involve testing its efficacy in mice or other models infected with malaria. Clinical research is less extensive compared to traditional and preclinical studies. Some clinical trials have looked into the efficacy and safety of andrographolide or *Andrographis paniculata* extracts in treating malaria, however the results still debated.[5] Current research continues to investigate the potential of andrographolide in malaria treatment, especially as a complement to existing therapies or as a lead compound for new drug development. Despite promising results, there are challenges including bioavailability, dosage, and the need for more robust clinical evidence to support widespread use.[6,7] Andrographolide has a rich history in traditional medicine and shows promise as an antimalarial agent. However, further research is necessary to properly understand its efficacy and safety in clinical settings.[8,9] The current level of andrographolide research is in vitro and in vivo testing; however, no clinical trials on humans or malaria patients have been conducted. Previous studies have shown that andrographolide possesses antimalarial properties. However, there are no studies that describe the potential andrographolide therapy to prevent plasmodium resistance to antimalarial. We are interested in reviewing andrographolide from its advantages in pharmacological effects, its advantages can be as a complement, not resistant, as an anti-inflammatory which does not suppress the immune system, even as an immunomodulator, so it is very useful as an antimalarial. This systematic review aims to evaluate andrographolide's potential in preventing plasmodium resistance.

MATERIALS AND METHODS

Search Strategy

This systematic review is carried out to collect some reference data related to the topic research used in journals about potential of andrographolide from *Andrographis paniculata* has been studied previously as a medicine antimalarial against Plasmodium sp., from various sources national and international. Process data collection by searching reference journals through several databases like Google scholar, ScienceDirect and PubMed. Journal used as a reference is the journal with time span of the last 10 years (2014- 2024) using keyword: Andrographolide, *Andrographis*, malaria, plasmodium, Mus musculus with several method of study includes in vivo, in vitro, in silico and Randomized control trial. Research question: Is andrographolide possible as new antimalaria candidate and prevent antimalarial resistance?

Inclusion Criteria

Articles that have been used in this study were to meet inclusion criteria based on PICO method Population in question: human, *Mus musculus*, mice, mouse. Intervention of interest: Plasmodium infection, Andrographolide treatment, Andrographis paniculata treatment. Control or comparator: distilled water, uninfected subject. Outcome: parasitemia, parasite eradication. The selected papers investigated at least three important metrics: (1) Andrographolide, *Andrographis paniculata*, (2) phytochemical compounds, and (3) pharmacological mechanisms involved.

Exclusion Criteria

We excluded review papers, manuscripts without abstracts, and those that did not fulfill our inclusion criteria. This study does not include studies that examined the link between Andrographolide and other illnesses.

Data Extraction and Management

We used a reference manager called Zotero to analyze the publications that matched the inclusion criteria before proceeding with our research. The information gathered included the (1) andrographolide form, (2) Type of study, (3) control (4) Analytical Method, (6) Mechanism Pharmacology.

Data Screening and Extraction

In this literature review, the results of several in silico, in vitro, in vivo, and clinical experiments that assessed the influence of Andrographolide on malaria were reported.

RESULTS

Search Result

Based on keywords searched in several databases to collect publication of potential Andrographolide as antimalarial, such as PubMed 9 article, Google scholar 732 articles, Science direct

Fig. 1. Andrographis paniculata plant (source: https://powo.science.kew.org/taxon)

4 articles. About 264 articles do not match with co-occurrence terms, 124 articles excluded such as review, case report, comment and opinion. Article matches with PICO were 76 then duplicated files were 10, Article excluded because of not match with all inclusion criteria were 31, so the studies included in review were 35 articles. The successful data extraction was shown in the flow PRISMA diagram displayed in Figure 1. The bibliometric mapping of reviewed articles is presented in Fig 2. The 35 reviewed articles were clustered into 6 groups, (see in Table 2).

The includes study most of study using *Andrographis paniculata* extract (AP) as treatment followed by AP tablet, and andrographolide, andrographolide derivate. Type of study mostly in vivo followed by RCT, in vitro, and in silico. Control mostly no treatment, analytical method to compare outcome mostly blood smear

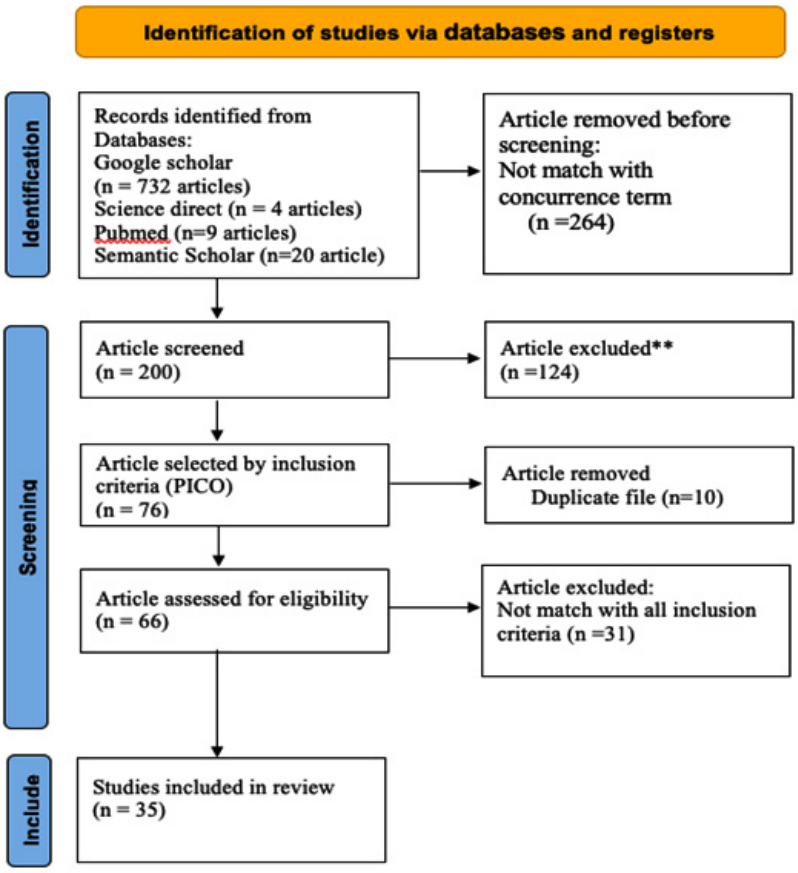

Fig. 2. Prisma analysis of studies included in review

and immunohistochemistry. Mechanism of andrographolide action as antimalarial agent was explained in discussion section.

Pharmacokinetic of Andrographolide

Absorption

Oral bioavailability of andrographolide is typically low. The reasons for andrographolide's low oral bioavailability are its rapid transformation, low aqueous solubility, high lipophilicity, and P-glycoprotein efflux. This is partly due to poor solubility in water and extensive first-pass metabolism in the liver, which reduces the amount of the compound that reaches systemic circulation.[57] After oral ingestion, andrographolide

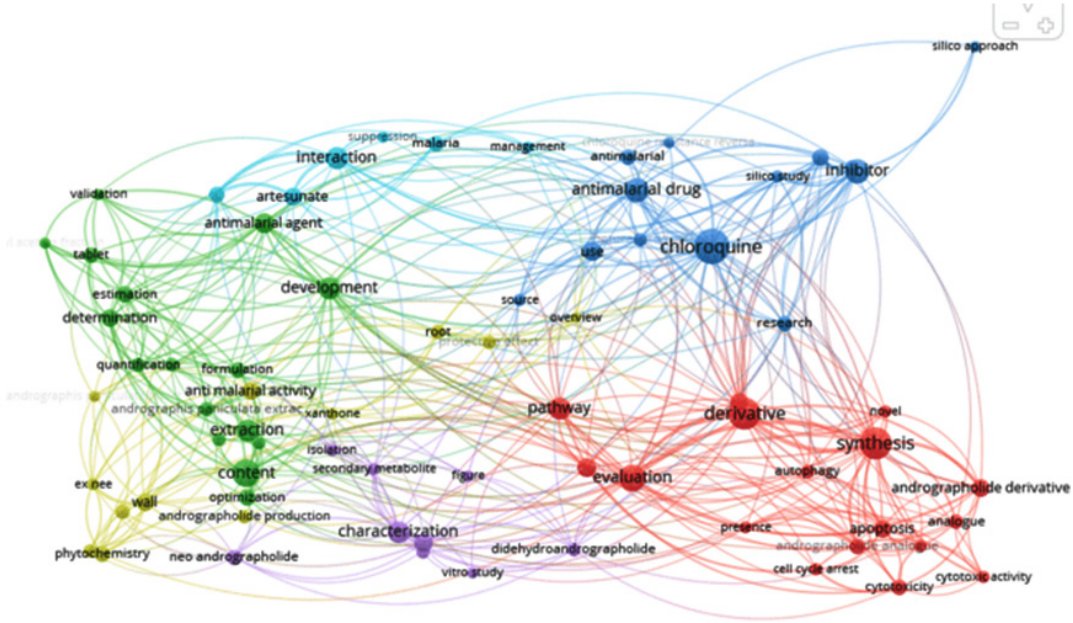

Fig. 3. Vos Viewer bibliometric mapping of reviewed articles

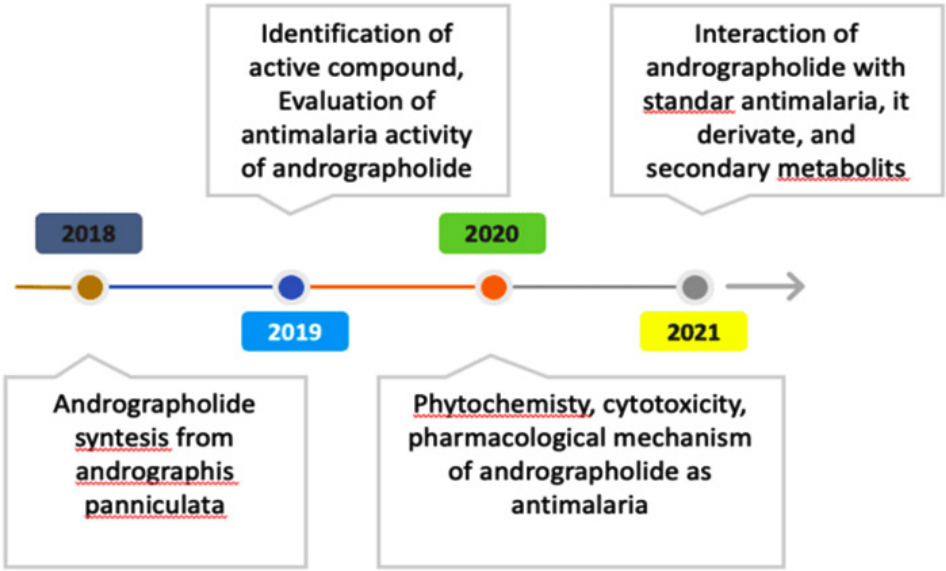

Fig. 4. Research timeline 2018-2021 of Andrographolide to be an antimalaria

is primarily absorbed in the gastrointestinal tract. However, because of its poor solubility, it might not be efficiently absorbed. Previous study explains andrographolide that given orally was absorbed in duodenum well. The pH level affects both the passive transport and carrier medium transport mechanisms involved in the intestinal absorption of andrographolide. The duodenum is the best absorption site, and the ileum is the best absorption site for dehydroandrographolid.[34,35] Combining solubilizing agents with a bioenhancer improved the oral bioavailability and pharmacokinetics of andrographolide; this implies that andrographolide formulations could benefit from this combination and could have further implications for clinical research.[36,37]

Distribution

With a half-life of roughly 25 minutes, andrographolide was rapidly absorbed into the blood from the gastrointestinal tract. After that, it bonded strongly to blood proteins and, in one to two hours, dispersed throughout tissues and blood. After 1.36 hours of dosing, the drug's maximal concentration was reached. Once in the bloodstream, andrographolide is distributed throughout the body. It can cross cell membranes

Table 1. The cluster mapping of reviewed articles

Cluster	Term	Thematic meaning
Cluster 1 (red color)	Analogue, Andro, Andrographolide Analog, Andrographolide Derivate, Antimalarial, Apoptosis, Autophagy, Cell Cycle Arrest, Cytotoxicity Activity, Cytotoxicity, Derivate, Evaluation, Growth, novel, pathway, presence, synthesis	This cluster describe how andrographolide synthesis from *Andrographis paniculata*, it analog and derivate, its cytotoxicity, how its pathway to be a novel antimalarial
Cluster 2 (green color)	*Andrographis paniculata*, andrographis, antimalarial agent, antioxidant activity, content, determination, development, estimation, ethyl acetate fraction, extraction, formulation, optimization, quantification, standard andrographolide, tablet, validation.	This cluster describes *Andrographis paniculata* has antioxidant activity, how it is extracted, formulated to enhance its bioavailability and optimization to be a standard andrographolide tablet. Estimated dose to be a standard antimalarial agent
Cluster 3 (blue color)	Antimalarial, antimalarial drug, chloroquine, chloroquine resistance, discovery, inhibitor, molecular docking, natural product, research, silico approach, silico study, source, use	Cluster 3 described phenomena of antimalarial resistance such as chloroquine and how silico study was simulated to discover a new antimalarial drug. Andrographolide was combined with a standard antimalarial agent to reduce the resistance.
Cluster 4 (yellow color)	*Andrographis paniculata*, Andrographis product, antimalarial activity, diterpene lactone, ex nee, overview, pharmacology, phytochemistry, protective effect, root, wall, xanthone	this cluster reviewed Andrographis product has antimalarial activity such as diterpene lactone, and it overview pharmacology phytochemistry, protective effect as antimalarial
Cluster 5 (purple color)	biosynthesis, characterization, dehydroandro-grapholide, figure, isolation, neoandrographolide, secondary metabolites, vitro study	This newest cluster described the biosynthesis and characterization of dehydro-andrographolide, neoandrographolide, their secondary metabolites that were proven by vitro study.
Cluster 6 (blue color)	artesunate, interaction, malaria, management, novel antimalaria diterpene lactone, suppression	This cluster described novel antimalaria diterpene lactone and it effect of parasite suppression and how it interacts with artesunate

Table 2. Result of Literature Review

Ref	Treatment	Type Of Study	Mechanism of Action
1	*Andrographis paniculata* (AP) tablet	RCT	Phase 1 clinical trial of AS201-01 tablets in healthy volunteers Is safe and no toxcicity found.
2	AP extract	in vivo	Suppression of parasite development. Reduced IFN-ã, increased IL-10 and IL-4, increased phosphorylated GSK3â (Ser9), decreased phosphorylated NF-êB p65 (Ser536), and phosphorylated Akt (Ser473)
3	AP extract	in vivo	decreased MDA levels and inhibited parasite growth by 81.97 ± 9.14%
4	AP extract	in vivo	Parasite growth suppression 80.35%.
5	AP extract	in vivo	Inhibit Macrophage migration inhibitory factor
6	AP extract	in vivo	Andrographolides not influence CCl4-induced oxidative stress in rats
7	AP extract	in vitro	Disturb Metabolism of plasmodium essential amino acid- metabolism
8	AP extract	in vitro	inhibit parasite growth
5	AP extract	in vivo	decrease macrofag inhibitory factor
9	AP extract	in vivo	Reduce the expression of IFN-ã and IL-10, no effect was seen in TNF-á level. Non-toxic or teratogenic in Foetal
10	AP extract	in vivo	Protects against liver and kidney damage, as well as hypoglycemia, during Plasmodium berghei infection
11	AP extract	in vivo	inhibit heme polymerization
12	AP extract	in vivo	suppressive, preventive, and curative actions, when combined with chloroquine, could provide an efficient alternative supply of herbal antimalarial medicines.
13	AP extract	in vivo	P. berghei 77.76% suppression
14	AP extract	in vitro	AP combination with Chloroquine or Artemisinin show more effective than single therapy of Chloroquine or Artemisinin in reducing parasitemia
15	AP extract	in vivo	Reduces plasma total cholesterol and triglyceride levels and prolongs survival time.
16	AP extract	in vivo	inhibited parasite's growth with inhibition range of 70.15% to 80.35%.
17	AP extract	in vivo	Parasite suppressive effect at a dosage of 50 mg/kg BW, inhibiting 73.48%. The 50% fatal dose (LD50) exceeded 2,000 mg/kg BW
18	AP Tablet	in vivo	reduce Cox-2 and prostaglandin expression on the placenta of P. berghei infected mice
18	AP Tablet	in vivo	suppressed P. berghei proliferation, raised TGF-â expression, decreased TLR-4 expression and apoptotic index in placental tissue, and decreased Cox-2 and prostaglandin expression
19	AP tablet	in vivo	AP help heme detoxification process of Plasmodium falciparum parasites
20	AP tablet	in vivo	Sambiloto tablet (AS20101) reduce the placental Chondroitin Sulfate A (CSA) Expression
21	AP tablet	In Vivo	AP tablet effective lowering TLR2 better that dihydroartemisinin piperaquine
22	AP tablet	in vivo	AP Tablet (AS201-01) Reduces TGF-â expression and inhibits parasite growth
23	AP extract	in vivo	In mice infected with P. berghei, parasitemia is reduced but erythrocyte and Hb levels are increased
24	AD	in silico	Muc-1 protein expression is decreased in mice infected with Plasmodium berghei ANKA in the medial colon.
25	AD	in silico	Andrographolide and its derivatives were the best interacted with plasmodiumplasmepsin I, II, and IV

26	AD	in vitro	indirect inhibitory effect on hemozoin production, decent growth inhibitory effect, but lower than chloroquine.
27	AD	in silico	improved binding interactions with plasmepsin I, II, and IV.
28	AD	in vivo	Andrographolide-curcumin lowering parasitemia (29%), extending the life span by 2-3 times
29	AD	in silico	Andrographolide and its derivatives bind most effectively to (PFGGPPS)
30	AD	in silico	inhibit parasite growth
31	Andrographolide	in vivo	inhibit a radical molecule's chain reaction, hence lowering the effects of free radicals
32	Andrographolide	in vitro	inhibits the plasmodium-induced permeation route and the ability of merozoites to penetrate new RBCs
33	Andrographolide	in vitro	inhibited parasitaemia but not as well as chloroquine phosphate

Abbreviation: IHC: immunohistochemistry, plasmodium PF geranylgeranyl pyrophosphate synthase (PFGGPPS), AD: Andrographolide derivate

Table 3. Pharmacokinetic Parameter of Andrographolide

Single dose			Ref.
PK parameter	Andrographolide		
T 0-24 h	60 mg (n=12)	120 mg (n=11)	53
CMAX (ng/ml)	72.1± 28.7	112 ± 33.4	54
T max (h)	0.80	0.80	55
AUC (0-24 h) ng-h/mL	210 ± 25.4	345 ± 63.5	56
AUC ((0-inf) (ng-h/mL)	210 ± 25.4	345 ± 63.5	53
MRT (h)	6.0 ± 0.45	5.3± 0.63	53
Vd/F (L/Kg)	9.0 ± 1.66	10.8± 1.6	54
Cl/F (L/Kh/Kg)	4.9 ± 0.84	6.0 ± 0.91	54
Talf-life (h)	1.3 (0.03)	1.2 (0.05)	55
Urine (To-48 h)			
Cumulative urine (ug)	372 ± 159	1.193 ± 553	55

*Abbreviations: Cmax, maximum plasma concentration; Tmax, time to reach maximum concentration; AUC, area under the plasma concentration–time curve; MRT, mean residence time; Vd/F, the apparent volume of distribution; Cl/F, the apparent clearance; Half-life, elimination half-life.T: time

and enter various tissues, but its distribution is influenced by factors like its lipophilicity (fat affinity) and the presence of transport proteins. Andrographolide is known to bind to plasma proteins, which can affect its free concentration in the blood and its distribution to tissues. Protein binding can also influence its pharmacokinetics and pharmacodynamics. Andrographolide can penetrate different tissues, including the liver, lungs, and kidneys. The extent of its distribution to these tissues can be influenced by its chemical properties and the blood flow to the tissues. The kidney, liver, spleen, and brain had the highest concentration of andrographolide (156.12 ng/g), according to the current study. The concentration of andrographolide in the lung and heart was almost the same. The values obtained for apparent C(max), T(max), elimination half-life, and total exposure (AUC(0-á)) were 115.81 ng/ml, 0.75, 2.45, and 278.44 ngh/ml, respectively.[38,39]

Metabolism

There hasn't been much focus on reports regarding andrographolide metabolism in people and rats. Andrographolide goes through phase I processes in the liver, which include oxidation, reduction, and hydrolysis. Liver enzymes, such as the cytochrome P450 enzymes, mediate these processes. These processes can modify the

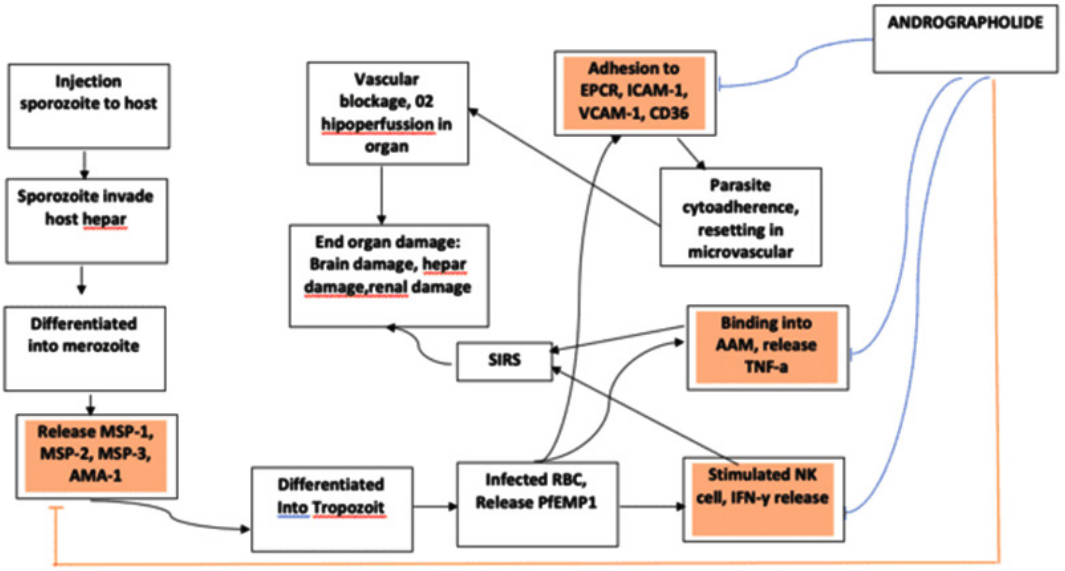

Fig. 5. Several possible mechanisms of andrographolide as antimalaria

Table 5. Estimated Andrographolide Dose and Toxic Effect

Dosage/duration/route	Experimental Model	Toxic effect	Ref.
10 mg/kg for 3 weeks	Human	No	65
500 mg/kg bw for 7 days i.p.	Mice	No	23
25–75 µM	Platelet	No	66
20 mg/kg bw for 60 days, oral	Rats	No	6
22–55 µg/kg, i.v.	Mice	Lower mortality	5
1 g/kg/day for 4, 6, and 8 weeks	Rat	No	20
100 mg/kg, i.p.	Mice	No	67
10 mg/kg, i.v.	Rats	No	57

chemical structure of andrographolide, potentially leading to the formation of various metabolites. Following phase I reactions, andrographolide may undergo phase II reactions, including conjugation processes like glucuronidation or sulfation. These reactions add polar groups to the molecule, which generally increases its water solubility and facilitates its excretion. One common metabolic pathway involves the hydroxylation of andrographolide, leading to the formation of hydroxylated metabolites. Conjugation reactions produce glucuronides and sulfates, which are more water-soluble and can be readily excreted.[59] Andrographolide's three categories of metabolic adducts include glucuronide conjugates, creatinine adducts, and sulfonic acid or sulphate type adducts. Even so, two creatinine adducts and seven glucuronide conjugates have been found as well as sulfonic acid or sulphate type adducts. Deoxy-sulpha-andrographolide, which possesses anti-inflammatory properties, has been found to be the main metabolite of andrographolide in rats.[57] Roughly 10 metabolites of andrographolide have been identified and described; these are mainly sulfate compounds and sulfonic acid adducts. Four novel urinary metabolites of andrographolide in humans have been identified: andrographolide-3-O-sulfate (M-1), isoandrographolide-3-O-sulfate (M-2), 14-deoxyandrographolide-3-O-sulfate (M-3), and 14-deoxy-12-(cysteine-S-yl)-andrographolide-3-O-sulfate (M-4). [40]

Dose-dependent effect was seen when the plasma concentrations of the four main parent compounds increased twofold upon a twofold increase in andrographolide dosage; 1) andrographolide, 2) didehydro-andrographolide, 3) Neo andrographolide, and 4) deoxy-andrographolide, as well as their conjugated metabolites. The majority of diterpenoids that have been found are conjugated metabolites, while the remainder are bio transformed in part via a phase II metabolic route that decreases the parent molecules in the plasma. The hepatobiliary system and urine elimination are the next pathways via which these compounds are eliminated. According to the safety evaluation's findings, people occasionally suffered mildly intense, infrequently occurring adverse events that were reversible to the baseline. Since there is no study on the use of andrographolide administration in humans with hepatic or renal impairment, safety considerations should be made based on the specific health conditions of each patient.

Excretion

Andrographolide and its metabolites are mainly excreted via the bile into the intestines. This process involves the liver secreting andrographolide and its conjugated forms (such as glucuronides and sulfates) into bile, which is then stored in the gallbladder and released into the small intestine. Once in the intestines, andrographolide and its metabolites are eventually eliminated in the feces. This is the primary route of excretion for andrographolide, especially since the liver is a major organ involved in its metabolism. Some of andrographolide's metabolites are excreted via the kidneys into the urine. Although this is a secondary route of excretion compared to biliary elimination, it can still contribute to the overall clearance of the compound from the body. In small amounts, andrographolide or its metabolites might also be excreted through sweat or saliva, though these routes are not significant compared to biliary and renal excretion. One likely biotransformation process for andrographolide is glucuronidation, as evidenced by the excretion of glucuronide conjugates in feces and urine.[61] All of the metabolites were characterized as glucuronide conjugates was found in human urine after oral administration of andrographolide. The structures were identified as follows: andrographolide-19-O-B-D-glucuronide, isoandrographolide-19-O-B-D-glucuronide, 14-deoxy-12-hydroxy-andrographolide-19-O-B-D-glucuronide, andrographolide-19-O-[6B-methyl-B-D-glucuronide], 14-deoxy-12(13)-en-andrographolide-19-O-B-D-glucuronide, 14-deoxyandrographolide-19-O-B-D-glucuronide, and 3-oxoandrographolide-19-O-B-D-glucuronide, respectively.[35,41–43]

Pharmacodynamic of Andrographolide as antimalaria

Site of andrographolide action also have been evaluate because of its variation. Studies reviewed above show andrographolide dominantly act in blood, liver, spleen and placental tissue.

Estimated dose in preclinical study

Andrographolide dosages in animal research are frequently determined by body weight and might vary from milligrams to several milligrams per kilogram of body weight each day. For instance, doses could vary from 10 mg/kg to 100 mg/kg or more, contingent upon the intended outcome and the animal species being employed. The quantities of andrographolide in research carried out in vitro on cell cultures can differ considerably. Concentrations typically range from micromolar (μM) to millimolar (mM) levels, depending on the experimental setup and the specific cell type being studied. The route of administration also influences the effective dose of andrographolide. In preclinical studies, andrographolide can be administered orally, intravenously, or through other routes depending on the experimental design.[12] Various dose in experimental model was presented in table 2.[55] Many studies use standardized extracts containing a specific percentage of andrographolide (for example, 10% to 30% andrographolide content. A common dosage might be an extract providing 300 mg to 600 mg of andrographolide per day.[55]

DISCUSSION

Suppression of Parasite Growth

Research on andrographolide as an antimalarial agent has been progressing, early research on in-vitro study demonstrated that andrographolide exhibits inhibitory effects on Plasmodium falciparum. Andrographolide could inhibit the parasite's growth at various stages of its life cycle. Andrographolide work inhibit

merozoite invasion to red blood cell, prevent gametocyte formation, induce apoptosis in infected erythrocyte, andrographolide also prevent energy production like ATP.[32] This fact supported by in vivo study, malaria animal models have provided further evidence of andrographolide's antimalarial activity by using *Plasmodium berghei* as analog *Plasmodium falciparum*.[4] Widyawahruyati has carried out trials of administering *Andrographis paniculata* tablets to healthy people to test its toxicity and safety. Makmur have also conducted phase 1 clinical trials on 69 malaria patients who were given tablets made from *Andrographis paniculata* ethanol extract. The result was 94.2% treatment success and no side effects were found. Lack of study of using andrographolide in human malaria, produce new perspective study in randomized clinical trial andrographolide on human malaria. While preclinical data is promising, clinical trials are necessary to confirm the safety and efficacy of andrographolide in humans. There have been preliminary human studies, but more extensive clinical trials are required to establish its use as a standard anti-malarial treatment.

Studies on the safety and toxicological profile of andrographolide are crucial for its development as a therapeutic agent. Research so far indicates that andrographolide has a favorable safety profile, but comprehensive toxicological studies are necessary to ensure its safe use in humans. Combination Therapies studies have explored the potential of combining andrographolide with existing anti-malarial drugs to enhance efficacy and reduce resistance. Studies suggest that andrographolide can work synergistically with drugs like chloroquine and artemisinin, potentially offering a more effective treatment option.[44] The correct combination formulation between andrographolide and chloroquine or artemisinin has not yet been found so future research can focus on adjusting the appropriate combination dose.

Inhibits Plasmodium Key Enzyme

Most antimalarial drugs work on plasmodium folic acid synthesis. The molecular mechanism of action of andrographolide is currently still a question. Although it can suppress parasite growth, the molecular mechanism is still not understood. Future research must elucidate how andrographolide works at the molecular level. Studies could focus on identifying specific molecular targets and pathways involved in its activity against Plasmodium parasites. Plasmodium falciparum lactate dehydrogenase (PfLDH) or dihydrofolate reductase (PfDHFR) has been proposed as a possible molecular target for antimalarial drugs due to the parasite's dependence on glycolysis for energy synthesis. Since P. vivax, P. malariae, and P. ovale's LDH enzymes (pLDH) and PfLDH are around 90% comparable, it would be beneficial to find novel anti-pLDH medications, especially those that work against P. falciparum, the most virulent form of human malaria. Until yet, no research has been conducted on the influence of andrographolide on the process of suppressing plasmodium enzymes such as plasmodium falciparum lactate dehydrogenase, although various in silico studies have revealed that andrographolide has a high affinity for the plasmodium falciparum enzyme receptor. Several homologues of aspartic proteases, including plasmepsin I, II, and IV, are encoded by the malaria parasite and are crucial for the breakdown of host erythrocyte hemoglobin within the parasite feeding vacuole.[27] Andrographolide has been shown to disrupt critical enzymes essential for the survival and proliferation of Plasmodium parasites, such as Plasmodium falciparum lactate dehydrogenase (PfLDH) and Plasmodium falciparum dihydrofolate reductase. Phytochemicals from *A. paniculata* were molecularly docked with both native and mutant DHFR. The active site residues of both wild and edited strains are highly receptive to phytochemicals and interact with them. For both strains, 14-Deoxy-11-Oxoandrographolide showed a binding energy of more than 10 kCal/mol.[45]

Andrographolide was proven previously to inhibit dihydrofolate reductase-thymidylate synthase and Plasmodium falciparum M1 alanyl aminopeptidase. Riboflavin, D-glutamate and D-glutamine phenylalanine, glutathione, proline and arginine, arginine biosynthesis, citrate cycle, glycolysis or gluconeogenesis, pyruvate, and alanine, aspartate, and glutamate metabolism are all disrupted. Andrographolide effectively inhibits parasite lactate dehydrogenase (pLDH). Other studies found that antimalarial andrographolide activity could affect the parasite antioxidant defense system, as seen by a drop in GSH levels and the thioredoxin reductase (TrxR) enzyme's activity. Andrographolide has also been shown to reduce

MDA levels and inhibit Dihydrofolate reductase (DHFR). It also inhibits Plasmodium falciparum Dihydroorotate Dehydrogenase (PfDHODH). An essential component of the processes that produce amino acids and nucleic acids is dihydrofolate reductase (DHFR). Using NADH as a cofactor, DHFR catalyzes the transformation of dihydrofolate into tetrahydrofolate.[46–48] Research on the mechanism of andrographolide to inhibit DHFR is still open and needs to be confirmed with silico study and in vivo study.

Induce ROS Accumulation inside Plasmodium

Andrographolide has been shown to promote the formation of reactive oxygen species (ROS) by Plasmodium parasites. ROS are very reactive chemicals that can harm biological components like lipids, proteins, and DNA. An increase in ROS can cause oxidative damage to several cellular structures and chemicals within the parasite. This includes damage to the parasite's mitochondrial and endoplasmic reticulum membranes, which are critical for its survival and replication. Plasmodium parasites have their own antioxidant defense systems, including enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which help neutralize ROS and protect the parasite. Andrographolide may inhibit these antioxidant defenses, leading to a higher level of oxidative stress and ultimately to the death of the parasite. Glutathione is a critical antioxidant that helps in maintaining the redox balance within cells. Andrographolide may reduce glutathione levels in Plasmodium, thereby impairing the parasite's ability to cope with oxidative damage. The oxidative stress induced by andrographolide can interfere with crucial metabolic pathways in Plasmodium. This includes the disruption of the parasite's ability to synthesize essential molecules and perform vital functions, leading to reduced parasite growth and replication. The oxidative damage to proteins, lipids, and nucleic acids can be toxic to the parasite, compromising its cellular integrity and function. This can result in the parasite's death or a significant reduction in its ability to proliferate. Studies have shown that andrographolide can lead to increased oxidative stress markers in Plasmodium falciparum and other Plasmodium species. These studies often use various assays to measure ROS levels and oxidative damage indicators in treated parasites. The lack of evidence, how it works in malaria patients, has a contrary negative effect in hosts due to increasing oxidative stress status. Previous studies show Andrographolide can enhance the phagocytic activity of macrophages, improving their ability to engulf and destroy pathogens. It also influences the production of pro-inflammatory cytokines, which are crucial for initiating and sustaining immune responses. Andrographolide also affects dendritic cells, which are key antigen-presenting cells. By modulating their function, andrographolide can influence the activation and maturation of these cells, thereby affecting the overall immune response.[49,50] But how it works in malaria conditions has not been investigated yet.

To avoid parasite degradation by the immune system, plasmodium binds to the ICAM, VCAM, CD 36 receptors thereby blocking the capillaries. This capillary blockade will cause blood flow to the tissues to decrease, resulting in hypoxia and leading to organ death. Prediction of andrographolide in this case works to block the parasite's binding to ICAM, VCAM, CD 36, so that its binding to these receptors is released and blood flow to the tissue returns smoothly.59,62 Artemisinin cannot bind to the VCAM, ICAM and cd 36 receptors so it cannot prevent capillary blockade by parasites. andrographolide can bind to the ICAM, VCAM and cd 36 receptors thereby blocking the binding of these endothelial receptors by the parasite.[51,52] In the future, andrographolide can be combined with artemisinin to reduce resistance.

Modulating Immune System

Andrographolide's anti-inflammatory action in malaria can occur via a variety of mechanisms, including: reducing the expression of TNF-a, IL-6, VCAM-1, ICAM-1, VEGF, NF-kB, IFN-y, CD4+, COX-2. By reducing inflammation, andrographolide can alleviate some of the symptoms associated with malaria, such as fever and systemic inflammatory response syndrome. It also increases IL-10 expression, the role of anti-inflammatory cytokine. Andrographolide has been demonstrated to regulate the production of numerous cytokines, including IL-6, IL-12, and TNF-á. These cytokines are important in generating inflammation and directing the immunological response, particularly during malaria infection. It also affects the amounts

of anti-inflammatory cytokines like interleukin-10 (IL-10), which aid in the control and resolution of inflammation.[53] Andrographolide can impact the Th1/Th2 balance, which is critical for determining the type of immune response. It may promote a Th1 response, which is more effective in dealing with intracellular pathogens like viruses and certain bacteria. It can influence the function and development of regulatory T-cells, which help in maintaining immune tolerance and preventing excessive immune responses. But how it affected Th1/Th2 response in malaria infection have not been investigated yet. This immune profile has not been modeled in malaria infections treated with andrographolide yet.

Andrographolide has the ability to block the NF-êB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling system, which is crucial for regulating immunological responses and inflammation. Andrographolide aids in reducing excessive inflammation by altering this pathway. To completely grasp its influence and efficacy in various immune-related disorders, particularly in malaria infection, more investigation and clinical trials are needed. Unfortunately, research has not yet been done on the location of andrographolide within Plasmodium parasites and host cells, changes in Plasmodium treated with andrographolide's ultrastructure, or identification of potential cellular structure damage. There is currently no information on how andrographolide altered Plasmodium signal transduction pathways. However, it has been shown that andrographolide inhibits the transcription factor nuclear. The immunological system and inflammation are influenced by Nuclear factor kappa B (NF-kB). Because andrographolide reduces NF-kB activity, fewer adhesion molecules and pro-inflammatory cytokines are expressed. Andrographolide inhibits the binding of NF-5ØßB oligonucleotides to nuclear proteins, resulting in antimalarial activity. This inflammatory process takes place in the human cerebral endothelium. TGF-â expression rose, but TLR-4 expression and apoptotic index decreased. Reduce levels of IFN-ã, IL-10, and TNF-á. Andrographolide effectively reduces Muc-1 expression in the intestines of Plasmodium-infected mice. It also decreases Cox-2 and prostaglandin expression. Decreasing expression of Toll-Like Receptor-4 (TLR-4) and placental apoptosis index. Evaluating its teratogenic effect, an Andrographis tablet did not change the placental Chondroitin Sulfate A (CSA) expression in pregnant mice.[18]

Prevent Heme Detoxification

Andrographolides are active in inhibiting heme polymerization. Interact with plasmepsin I, II, and IV to inhibit hemozoin production. Heme fractionation and â-hematin formation studies were used to assess the influence on hemozoin formation. Furthermore, it enhanced the reduction of hemozoin production generated by chloroquine and showed an indirect inhibitory effect on hemozoin development within the parasite. According to the study, its chloroquine resistance reversal effect could be due to a reduction in chloroquine accumulation or an effect on the parasite's biological activity. Andrographolide was incubated with uninfected RBCs to determine its effects on form, integrity, and osmotic fragility. It was cultivated with plasmodium-infected red blood cells to see how it affected parasite-induced permeation routes. The effect on merozoites' ability to enter fresh RBCs was studied using a merozoite invasion experiment. Andrographolide was revealed to be safe to RBCs at levels comparable to its therapeutic level against plasmodium. However, its inertness was diminished at higher concentrations.[32]

Artemisinin resistance is most commonly related with mutations in the kelch13 gene, which is part of a gene cluster that regulates protein breakdown. These alterations influence the parasite's reaction to the treatment by changing its ability to undergo a critical stage of its lifecycle, resulting in slower parasite clearance from the bloodstream. Specific mutations in the kelch13 gene affect the protein's stability and function, reducing the parasite's capacity to respond to artemisinin and its derivatives. This prolongs the parasites' survival in the presence of the medicine. Previous research demonstrated that andrographolide can increase deposit reactive oxygen species inside parasite mitochondria, induced parasite damage and death. Its action analogue with artemisinin action, these phenomena make andrographolide can work synergistically with artemisinin as antimalarial and need to be investigated their role in prevent artemisinin resistance by inhibiting or preventing mutation of kelch 13 gene. In silico study must be done to open fact of possible andrographolide binding with kelch 13 protein.[54–56] By binding

andrographolide with its receptor possible of gene kelch 13 mutation can be prevented.

Heme detoxification is a crucial process in organisms that degrade hemoglobin, particularly in blood-feeding parasites like Plasmodium. These microbes break down hemoglobin to liberate heme, a potentially hazardous chemical that can damage cellular membranes and produce reactive oxygen species (ROS). To mitigate this toxicity, these organisms have developed mechanisms to detoxify heme. Here's how heme detoxification typically occurs: (1) Heme Aggregation into Hemozoin: The malaria parasite Plasmodium ingests host red blood cells and digests hemoglobin within its digestive vacuole. The parasite is poisoned by the free heme (ferriprotoporphyrin IX) that is released during this digestion. The parasite changes heme into hemozoin, or malaria pigment, an insoluble crystalline form, in order to detoxify it. This process involves the self-assembly of heme molecules into a crystalline structure, where the toxic iron center is sequestered within the crystal, reducing its ability to catalyze the formation of ROS. Mechanism of hemozoin formation is not fully understood, but it is believed to involve both spontaneous crystallization and possibly the action of parasite-derived proteins or lipids that facilitate the aggregation. The carboxylate groups of the heme molecules are thought to interact with each other, leading to the formation of dimers, which then stack together to form the hemozoin crystal. By sequestering heme into hemozoin, the parasite avoids the toxic effects of free heme, allowing it to continue digesting hemoglobin and proliferating within the host. In silico docking must be investigated further to analyze binding of andrographolide with ferriprotoporphyrin IX. Further research is also done in vivo to confirm possible andrographolide prevent heme detoxification by plasmodium with competitive binding with ferriprotoporphyrin IX.[19]

Autophagy Regulation

Andrographolide has shown potential in inhibiting autophagy in Plasmodium, the genus of parasites responsible for malaria, through several mechanisms. First inhibition of Autophagy-Related Proteins and autophagy related gene (ATG), Andrographolide may interfere with key autophagy-related proteins (such as LC3 and Beclin-1) and autophagy related gene, preventing the formation of autophagosomes, which are essential for the autophagic process. Second modulation of Signaling Pathways. The compound can affect signaling pathways that regulate autophagy, particularly by inhibiting the mTOR pathway, which, while usually promotes autophagy, can have varying effects in the context of Plasmodium infection. Third, disruption of Metabolic Processes, by altering metabolic pathways within the parasite, andrographolide can create a stress environment that diminishes the effectiveness of autophagic degradation mechanisms. Fourth, Andrographolide may increase ROS levels, leading to cellular stress. In some contexts, this can impair autophagy, although the relationship between ROS and autophagy can be complex. Fifth, impairment of Host-Pathogen Interactions, the compound may disrupt the interactions between Plasmodium and the host cell's autophagic machinery, inhibiting the parasite's ability to evade host defenses. The role of andrographolide in the autophagy process is divided into 2 pathways, namely induction of survival cells and induction of death cells. In previous studies, it was proven that dehydro-andrographolide can trigger autophagy in cancer cells, so that the number of cancer cells in the body is reduced. The role of andrographolide in the plasmodium autophagy process has never been reported, thus opening up opportunities for research into the mechanism of plasmodium autophagy influenced by andrographolide.[57–58] By targeting these mechanisms, andrographolide can potentially weaken the Plasmodium parasite's survival and proliferation, making it a candidate for further research in preventing resistance of antimalarial treatment.

Until now, andrographolide preparations are still in the form of crude extract, granules, tablets and liquid, where the absorption capacity or bioavailability is very low when given orally due to first pass effect.[59-60] Future research should examine the route of administration such as injection and also andrographolide preparations, such as nanoparticles, liposomes, or other formulations, to improve the bioavailability and stability of andrographolide. These systems can enhance the drug's therapeutic effects and reduce side effects.[61] Open discussion on mechanisms of andrographolide to prevent anti-malaria resistance arise gap research and potential new research in

andrographolide to prevent antimalarial resistance. No research has been conducted on the potential of andrographolide to prevent Plasmodium resistance. Further research must be conducted in design to expose Plasmodium cultures to andrographolide and analyze the overcome of resistant markers. Cross-Resistance Studies also can be done to investigate whether andrographolide-resistant Plasmodium strains exhibit cross-resistance to other antimalarial drugs.

CONCLUSION

Andrographolide from *Andrographis paniculata* is a promising alternative treatment to prevent antimalarial resistance. The mechanism of action such as suppression of parasite growth, inhibit plasmodium key enzyme, prevent ROS production, prevent heme detoxification, modulating immune response and regulating autophagy. Make andrographolide potential to be a combination, adjuvant or single therapy for malaria. More in-depth research on how andrographolide works at molecular level to suppress the incidence of antimalarial resistance should be further investigated.

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This research does not involve any clinical trials

Authors' Contribution

Putu Indah Budiapsari: Conceptualization, Methodology, Writing – Original Draft; I Ketut Suryana: supervision and advice; Bagus Komang Satriyasa: review and editing; Dewa Ayu Agus Sri Laksemi: Data Collection, Analysis, Writing – Review & Editing; Dyah Kanyawati: Visualization, Supervision, Project Administration; I Made Agus Gelgel Wirasuta: concepting, Resources, Supervision.

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