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Pharmacological Study of *Andrographis paniculata* (Kalmegh) for their possible Antimalarial Activity with Emphasis on Resistance and Resistant Reversal

Jai Kumar Mishra[1], Kumud Upadhyay[2], Sharad Visht[3], Ramandeep Singh[4], Anita Kumari[5], Hareesh Dara[6], Amit Kumar Verma[7], Sunita Singh[8]

Abstract

Antimalarial effect of some medicinal plants found in Uttarakhand (India) is least explored by the scientific community locally. Advent of resistance to various antimalarial drugs by plasmodium made malaria more fatal and life-threatening disease. Therefore, present need is to invest more effort and interest in research for antimalarials from medicinal plants. *Plasmodium yoelii* nigeriensis (PYn) is multi drug resistance malaria parasite known for resistance to chloroquine (CQ), quinine, quinidine, amodiaquine, halofantrine, mepacrine, and mefloquine. The *P. yoelii* produces 100% infections in animals. Researchers in this study tried to understand the behavior of CQ -resistant plasmodium PYn with CQ, whole plant extracts of *Andrographis paniculata* (AP) with possibility of their resistance reversal. Wherever, 3–4 times CQ doses are not able to produce sufficient antimalarial effect in resistant PYn. Most of the pure plant extracts are also not able to produce minimal therapeutic response when given alone. Whereas, plant extract shows a better effect when given with minimal dose CQ than alone. AP whole plant hydroalcoholic (HA) extract doses 300 mg/kg when combining CQ 20 mg/kg shows the best effect among the other extracts alone. Parasitemia and red blood cells count parameters significantly improved. Hemoglobin, survival days and weight are benefitted with HA extract then ethanolic extract. Unexpectedly, HA extract at higher dosage 1000 mg/kg produce efficacy then 300 mg/kg dose group. Higher doses of HA extract causes some sort of negative effect on almost all selected parameters even though with ethanolic extract. Present approach of multi-drug dosage supports also supported here in the study with a thought of using antimalarial plant extracts during regular malaria treatment. Genesis of idea of referring standardized plant extract(s) in combination as oral dosage with prophylactic (travelers) malaria and malaria treatment or side-food for malaria will also possible. Positive results may pave a path for inclusion of herbal extracts or herbs as side treatment or resistance-breaker food for malaria.

Keywords: Malaria, Parasitemia, *Plasmodium yoelii nigeriensis*, Chloroquine resistant, Resistance reversal, *Andrographis paniculata*

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Introduction

Malaria is one of the deadliest and oldest diseases known to humankind for more than 2000 years that causes the death of 0.4–05 million individuals annually. Literature reveals that, this disease known to Egypt, China, and India. Malaria continues to be a devastating parasitic ailment. According to the World Health Organization (WHO) estimates, 228 million cases of malaria occurred globally. In the era of resistance especially multi-drug resistance, finding a new efficacious and potent molecule is always a challenge for Pharmacological science together with the absence of an efficient vaccine hastens the need for speedy and comprehensive antimalarial drug discovery and development. Researcher across the globe sees resistance threat in every molecule. Every drug, molecule whether new or old has resistance possibility. Children aged 5 years or less are the most exposed and affected by malaria. In 2018, children aged under 5 years are accounted for 67% (272,000) of all malaria deaths worldwide. 41% of the world's population lives in areas where malaria is transmitted, which clearly means the danger of spreading and resistance will be there for a longer time than expected. Approximate, 350–500 million cases of malaria occur globally, out of that approximate one million people die every year; most of them are underage children, which accounted for almost 2 deaths/min. Malaria was the fourth cause of death in children in developing countries (after perinatal) in 2002. Malaria caused 10.7% of all children's deaths in developing countries. There were an estimated 405,000 deaths from malaria globally, compared with 416,000 estimated deaths

[1]Department of Pharmacy, Uttarakhand Technical University, Dehradun, Uttarakhand, GISIPS, Dehradun, Uttarakhand, India

[2]Department of Pharmacognosy, Kumau University, Kumau, Uttarakhand, India

[3]Department of Pharmaceutics, DIT University, Dehradun, Uttarakhand, India

[4]Department of Pharmacology, Himachal Institute of Pharmacy, Paonta Sahib, Himachal Pradesh, India

[5]Managing Partner, M/S LFE, Dwarka 13, New Delhi, India

[6]Department of Pharmaceutics, Sree College of Pharmacy, Kakatiya University, Warangal, Telangana, India

[7]Department of Pharmacy, MJP Rohilkhand University, Bareilly, Uttar Pradesh, India

[8]Department of Pharmacy, BBD University, Lucknow, Uttar Pradesh, India

Corresponding Author: Jai Kumar Mishra, Department of Pharmacy, Uttarakhand Technical University, GISIPS, Dehradun, Uttarakhand, India. E-mail: jaikumarmishra83@gmail.com

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in 2017, and 585,000 in 2010. According to the WHO, African region accounted for highest 94% malaria deaths that accounted for 85% of the 1,80,000 fewer global malaria deaths reported in 2018 compared with 2010. Nearly, 85% of global malaria deaths in 2018 were concentrated in 20 countries in the WHO African Region and India.[1] Regardless of having much natural resources, these countries are still unable to manage mortality rate of malaria. Many researcher and reports suggest.

Mountains are the richest source of fauna and flora. Indian hilly State Uttarakhand and Himachal Pradesh are the source of worthy medicinal plants. Multiple evidences from literature of medicinal plants show their effectiveness on various diseases. Interestingly, the success of the antimalarial drug quinine from cinchona and discovery of artemisinin from qinghao, the most potent antimalarial drug till today are from "plant sources," has led to the study of plants as antimalarial agents. The ethnopharmacological approach for the search of new antimalarial agents from plant sources has proved to be more predictive.[2,3] In 1820, first antimalarial drug was quinine, isolated from the bark of Cinchona is one of the oldest and most important antimalarial drugs that are still used today.[4] In 1940, chloroquine (CQ) was synthesized and until recently, and this was the only drug, used for the treatment of malaria on regular basis.[5] Unfortunately, after an early success, the malarial parasite especially *P. falciparum* became resistant to CQ.[6] Treatment of CQ -resistant malaria was done with alternative drugs or drug combinations, which were rather expensive and sometimes toxic. Furthermore, these combinations were not always based on pharmacokinetic principles due to inadequate knowledge of metabolism and mechanism of action of most antimalarial drugs. Hence, several research groups are now working to develop new active compounds as an alternative to CQ, especially from artemisinin[7] from the Chinese plant *Artemisia annua*.[8] Therefore, plants may well prove to be the useful source of new antimalarial drugs in view of the success with the two important chemotherapeutic agents.

Resistance and Malaria

Resistance is the greatest challenges facing malaria control today. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world.[9] Population movement has introduced resistant parasites to areas previously free of drug resistance. The economics of developing new pharmaceuticals for tropical diseases, including malaria, are such that there is a great disparity between the public health importance of the disease and the amount of resources invested in developing new cures.[10,11]

Anti-malarial drug resistance has been defined as: "the ability of a parasite to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject. The drug in question must gain access to the parasite or the infected red blood cell (RBC) for the duration of the time necessary for its normal action."[12] In most instances this refers to parasites that remaining following on from an observed treatment. Thus, excluding all cases where anti-malarial prophylaxis has failed.[13] The techniques used to demonstrate this are *in vivo*, *in vitro*, animal model testing, and the most recently developed molecular techniques. Drug resistant parasites are often used to explain malaria treatment failure. However, they are two potentially very different clinical scenarios. The failure to clear parasitemia and recover from an acute clinical episode when a suitable treatment has been given and anti-malarial resistance in its true form. Drug resistance may lead to treatment failure, but treatment failure is not necessarily caused by drug resistance despite assisting with its development. A multitude of factors can be involved in the processes including problems with non-compliance and adherence, poor drug quality, interactions with other pharmaceuticals, poor absorption, misdiagnosis, and incorrect doses being given.[9] The majority of these factors also contribute to the development of drug resistance.

The first type of resistance to be acknowledged was to CQ in Thailand in 1957. The biological mechanism behind this resistance was subsequently discovered to be related to the development of an efflux mechanism that expels CQ from the parasite before the level required to effectively inhibit the process of heme polymerization; that is necessary to prevent buildup of the toxic byproducts formed by hemoglobin (Hb) digestion.[14] This theory has been supported by evidence showing that resistance can be effectively reversed on the addition of substances which halt the efflux. The resistance of other quinolone antimalarials such as amodiaquine, mefloquine, halofantrine, and quinine are thought to have occurred by similar mechanisms. Plasmodium have developed resistance against antifolate combination drugs, the most commonly used being sulfadoxine and pyrimethamine. Two gene mutations are thought to be responsible, allowing synergistic blockages of two enzymes involved in folate synthesis. Regional variations of specific mutations give differing levels of resistance.[9]

Mutation and their markers for resistance, in which PfKelch13 mutations, have been identified as molecular markers of partial artemisinin resistance. PfKelch13 mutations associated with artemisinin resistance are widespread in the GMS and have also been detected at a significant prevalence (over 5%) in Guyana, Papua New Guinea and Rwanda. In the case of Rwanda, the presence of PfKelch13 mutations does not affect efficacy of first-line treatment. In the WHO Western Pacific Region, artemisinin resistance has been confirmed in Cambodia, Lao People's Democratic Republic and Viet Nam through several studies conducted between 2001 and 2018. Treatment efficacy for *Plasmodium vivax* remains high across all countries where treatment failure rates are below 10%. In the WHO African region the efficacy rates of artemether-lumefantrine artesunate-amodiaquine and dihydroartemisinin-piperaquine for *P. falciparum* were more than 98%, and efficacy has remained high over time.[1] In the WHO South-East Asia Region, the presence of molecular markers of artemisinin resistance has been reported in Bangladesh, India, Myanmar and Thailand. With the exception of Myanmar, failure rates of *P. falciparum* to first-line artemisinin combination therapies were found to be above 10% and were as high as 93% in Thailand. For *P. vivax* most countries continue to demonstrate high efficacy of chloroquine (CQ), except for Myanmar and Timor-Leste.[1]

Our selected *Plasmodium yoelii* nigeriensis (PYn) is most used strain for study of impact of CQ resist. Antimalarial effect of drug; especially model used is stages active against liver cycle also as well as the drug active against erythrocytic stage of parasite.

Resistance Reversal

Resistance reversal is partially proven but very important concept. Study for current context because some literature stated about the possibility of resistance of all major drugs even it is possible with the

artemisinin and their derivatives. The reversal is possible with some very selecting drugs such as verapamil, cyproheptadine, and Vitamin C,[14] but practically reversing drug are to be used with quite high quantity. The reversing effect of verapamil on CQ resistance has been confirmed in numerous studies; however, its biochemical target(s) and mechanism of action in malaria trophozoites are unknown. Bray *et al*.[15,16] have shown that verapamil increases CQ accumulation in resistant parasites. Bioprospection of medicinal plants may disclose active compounds that may serve as leads to develop new drugs that interfere in disease process. Taking into consideration that our understanding of the scientific principles of these herbal drugs is still unsatisfactory, resulting in the limitation of their widespread use in patients, the herbal medicine is having lesser side effects. The present study is about the bioprospection with special reference to prospecting Antimalarial effect of *Andrographis paniculata* (AP) especially emphasis on resistance reversal in CQ resist plasmodium strain

Research Methodology

Selection of Plant(s)

Researcher was unable to find much references of plant used either for antimalarial effect on resistant plasmodium or resistant reversal study. Hence, selection of plant(s) and their screening carried out by Short survey with ayurvedic practitioner, folk and local healer. In this survey, researcher excluded the most abundant reference of cinchona and quinghao (Jwar-roth) as both are not found in Uttarakhand. Secondly, we used the most abundant plant used commonly by local practitioner in number of common ailments. Common plants studied by many research scholars for antimalarial usage is as below which are not studied on resistant malaria plasmodium yet.

Out of top recommended plants, we used AP to assess the antimalarial activity on CQ resistant plasmodium due to its well-known pharmacological activity on hepatoprotective, hepato-stimulative action,[17] and schizonticidal activity.[18] Activity related to liver is indeed our first requirement while choosing the plant due to prime target of parasite after entry into the blood is liver only. However, researcher is unable to find the relevant studies on resistant plasmodium.

Extraction and Yield

Plant material collected locally from area Dehradun Uttarakhand to Paonta Sahib (HP) India. Plant(s) identification and validated by NIPER SAS Nagar Mohali. AP reference number NIP-NPM-CD-215A. Further, plant material air dried at the temperature not exceedingly more than 40 ± 5°C in room temperature/oven. Crushed plant material whole plant of AP with three solvents as below for overnight.

i) Aqueous (Distilled water)
ii) Ethanolic (Absolute alcohol)
iii) Hydro alcohol (1:1 ratio of Distilled water: absolute alcohol)

Fallowing filtration and concentrated by Rota vapor, followed by drying in oven to obtain a dry solid mass form. All extract preserved in 2–8°C till the end of the experiment.

Preparation of Doses

Water soluble extract oral dosage form prepared in RO water. And, ethyl alcohol/ hydro-ethyl alcohol extract dosage form prepared in 0.7% of Carboxy Methyl Cellulose solution to maintain the uniformity of the dose. Preserved the all the extract dosage in 2–8 °C till end of experiment.

Animal and place

Animal used for the study is Mice *(Musmusculus)*, strain swiss albino of either sex. Either male or female in one group. All animals are Outbred Swiss mice of either sex, weighing 25–45 g, and maintained on commercial pellet diet and water *ad libitum* under standard housing conditions. Place of work Deshpande Laboratories Pvt. ltd., Neelbud, Bhopal, Madhya Pradesh India. CPCSEA approval 1410/C/11/CPCSEA.

Test model - Modified Peter's and Rane's test

We modified Peter's (4 days suppressive test) and Ranes's test to assess the anti-schizonticidal activity of extracts in sporozoite-induced infections with rodent malaria parasite PYn in swiss mice. Methods as described by Peter *et al*.[19] and Ryley and Peters with modifications.[20] Instead of following regular screening on daily basis start from day 1, we screened the parasitemia on day 4 and day 7 for all animal experiment groups. However, basic suppression or curative principle of herbal extract does not change just other than observation of parameters. Standard ethical guidelines on handling and use of experimental animals were followed during the study. Parasite inoculation Passaging had made by the donor mice who had parasitemia near to 40–50%. Proper dilution of infected RBCs up to 10^6 cells in 0.2 ml of TCA buffer to infect mice through intraperitoneal route. Randomly divided infected mice were divided in respective groups. Treatment was started as immediate as possible after inoculation of mice. Parasite inoculation day called Day 0 or D0. Dosing 4 days on daily basis. After 4 days, blood from tail was taken to assess the parasitemia and percentage inhibition. Further to this, we follow assessment of other parameters on D0, D4, D7, D14, D21 and D28.

Material Used

List of material used and its Source

Dosing and sampling

Dosing frequency is once in a day for 4 days. Sampling frequency day 0, day 4, day 7, Day 10, day 14, day 21, and day 28. Parameter studied was survival days, RBCs count, Hb percent Optical Density (OD), Parasitemia and weight of animals.

Parameter

Major parameter for study is

(1) Survival time
(2) Parasitemia level
(3) RBCs count
(4) Hb percent OD
(5) Body weight.

Survival days

Measured usually on day to day basis. Data collected and monitored on D4, D7, D10, D14, D21 and D28.

Parasitemia

Parasitemia used as a measurement of parasite load in the organism and an indication of the degree of an active parasitic infection in the blood. Counting followed after slides preparation by a drop of blood taken from tail of the mice. After drying of slide for 15 min used methanol to fix the blood layer. Further to this, giemsa stain (5% solution) used for 50 min to color the smear. Washed the slides with water before drying.[21] We are used oil immersion method for read stained slides under 200×.

Parasitemia = Number of infected RBCs/total no. of RBCs × 100

RBC Count

Parasite mainly affects the blood system which makes RBC count an important parameter in this study. RBCs count by hemocytometer or neubouers chamber method. Diluted blood of experimental mice in the ratio of 1:200 with RBC diluting fluid helps in RBC count.

Hb % OD

Hemoglobinometry is the measurement of the concentration of Hb in the blood. Hb estimation is one of the common screening tests for the diagnosis of Anemia. For determination of Hb, we used standard kit method containing Drabkins diluting fluid with ratio of 1:200 fresh blood of animal. Diluted blood is analyzed for OD by Spectrophotometery at 540 nm with the help of 96 well plates.

For mice the normal value is 16.13g/dl (OD for standard was 231). Formula for OD to Hb conversion Hb g/dl = OD of Test/OD of Std. (231) * Hb level in g/dL (16.13)

Note: In our experiment, we comprise OD of Hb in all results. Conversion factor value is 0.0706, which needed to multiply in each test value to know the Hb g/dL. Therefore, we used Hb percentage OD without conversion factors.

Body weight

Body weight is a considerable factor in our study as a simple change in hematology, Physiology even in some psychology changes body weight easily. Recorded in grams.

Results

CQ Dose Selection on CQ Resistant Plasmodium

During the study of this group, although, standard and recommended dose to achieve anti-malarial action of CQ is 5 mg/kg. Which means, plasmodium used by us is clearly CQ resistant. We find that, dose 20 mg/kg do shows some efficacy as well as CQ 25 mg/g on CQ resistant plasmodium. Even in the higher groups of CQ ≥30 mg/kg is just uneventually deviate from a particular direction. Doses which is 4–8 times higher than the normal therapeutic dose able to cure the animal. However, both groups do not produce significant action comparatively. *PYn* our plasmodium strain showing inefficacy even at 4 times higher dose then the commended sensitive strain i.e. dose 5 mg/kg. Result for all 5 pre-defined parameters hemglobin % OD, Parasitemia, RBC count, body weight and survival days depicted prominence effects at selected doses. This helps us to select dose group CQ 20 mg/kg to combine with the selected plant(s) AP which are individually ineffective in another experiments. To understand the possibility of synergistic effect or possibility of resistance reversal activity combine with CQ20 mg/kg dose; if, improves results of all parameter toward antimalarial effect and longevity.

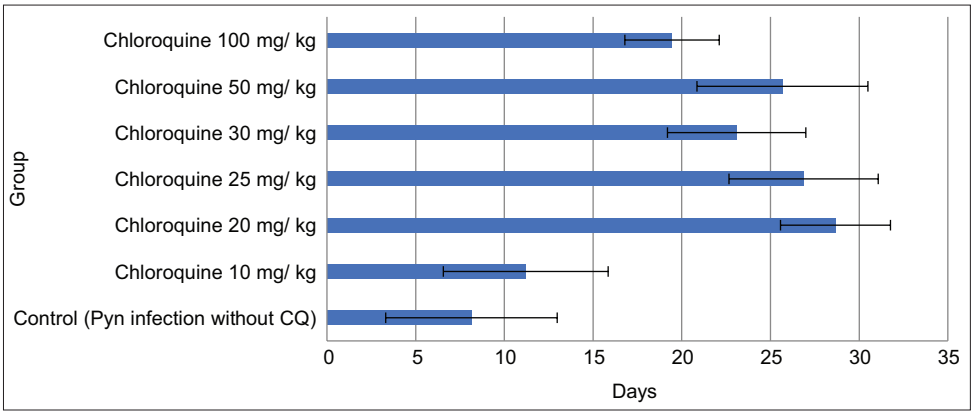
Antimalarial Study of AP Whole Plant Extracts with CQ 20 mg/kg

AP whole plant extract shows negligible effect on CQ resistant *Plasmodium yolii nigeriansis* (PYn) when given alone without CQ. We selected one lower dose and one higher dose of 300 mg/kg and 1000 mg/kg. Hypothesis is to test either of CQ-20 mg/kg and plant extract 300 mg/kg and or CQ-20 mg and plant extract 1000 mg/kg produces better antimalarial activity which either means potentiate the pharmacological effect or possibly reversal of resistance in PYn. Unfortunately, in the same series of experiments where we tested 5 plants and their multiple parts extracts. We find inefficacy of aqueous extract then hydroalcoholic (HA) or ethanolic extracts.

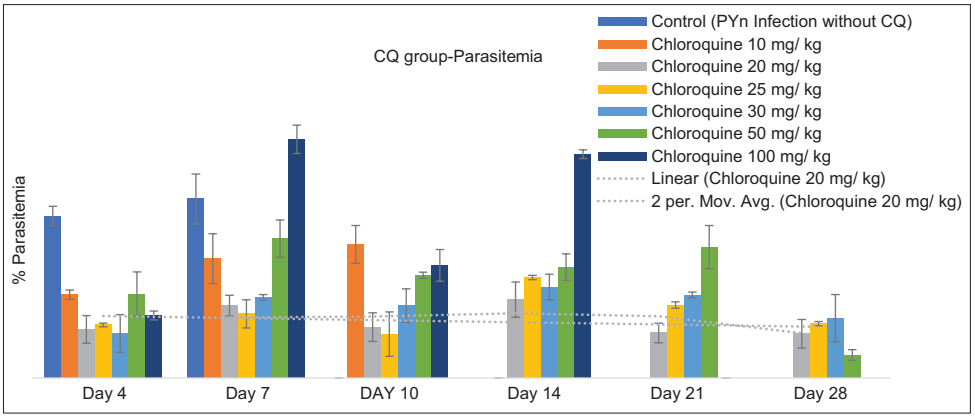
Discussions

First, we established the dose of CQ on resistant plasmodium within the local lab setup, metabolism of animals available and plasmodium strain in virulence. After finding a CQ dose; on which resistant plasmodium strain was neither effective nor totally ineffective will help to understand the possibility of reversal of resistance when combine with plant extract; if plant extract(s) produces potentiation or resistance reversal. We tried pure and crude plant extract individually. In the process of CQ dose selection, we tried 2–20 times higher doses of CQ to resistant plasmodium PYn and considered 5 mg/kg as a standard dose for CQ. Interestingly, we found CQ dose below 20 mg/kg is totally ineffective and above this does results are highly unreliable like no efficacy of CQ on multi drug resistant PYn. During the study, we found that most of the plants or their crude extracts were not able to produce antimalarial effect on our CQ resistant plasmodium strain. Results in trial were insignificant with single plant extract.

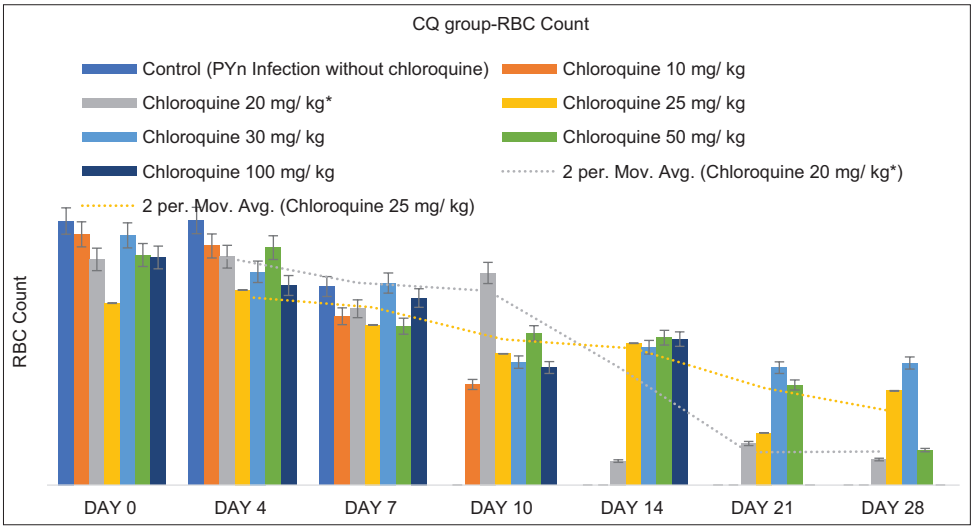
AP *is* a very versatile and widely used medicinal plant found abundant in India and in southeast Asia. Many ethnological and ethno-pharmacological studies indicate its uses as medicinal plant. During the study of AP whole plant extract effect with CQ, results were quite interesting and hopeful. In general, all extracts including Aq., HA and ethanolic extracts are capable to produce elevated and better results with CQ. Results of AP whole plant HA extract at lower dose 300 mg/kg is very promising for almost all the pre-decided parameters. Parasitemia and RBC count primarily shows best results followed by Hb and survival days. Results of higher doses 1000 mg/kg of HA extract are also hopeful for us. Furthermore, results of AP whole plant ethanolic extract higher dosage are hopeful again. Unexpectedly, higher doses 1000 mg/kg of HA extract produces suboptimal effect on parameter like Parasitemia and Hb OD then lower doses 300 mg/kg of HA. Which means higher doses either produce toxicity or least effective on efflux of CQ from the PYn, so degree of resistance reversal is reduced. Beyond our expectation from current or earlier experiments, higher doses 1000 mg/kg of aqueous extract AP whole plant were also able to show the significant effect on parasitemia, RBC count and Hb parameters. Effect from both type of plant extract is mostly not expected in most of the plant extracts. In general, pharmacological effect either exerted by polar compound (Aq. Or HA extracts) or by non-polar compounds. This



Graph 1: Survival days for chloroquine group



Graph 2: Parasitemia chloroquine group

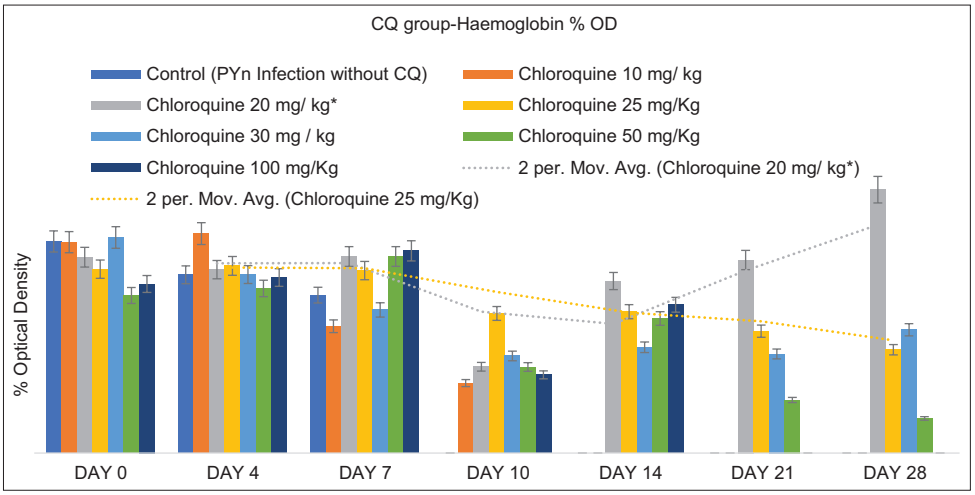


Graph 3: Red blood cell count for chloroquine group

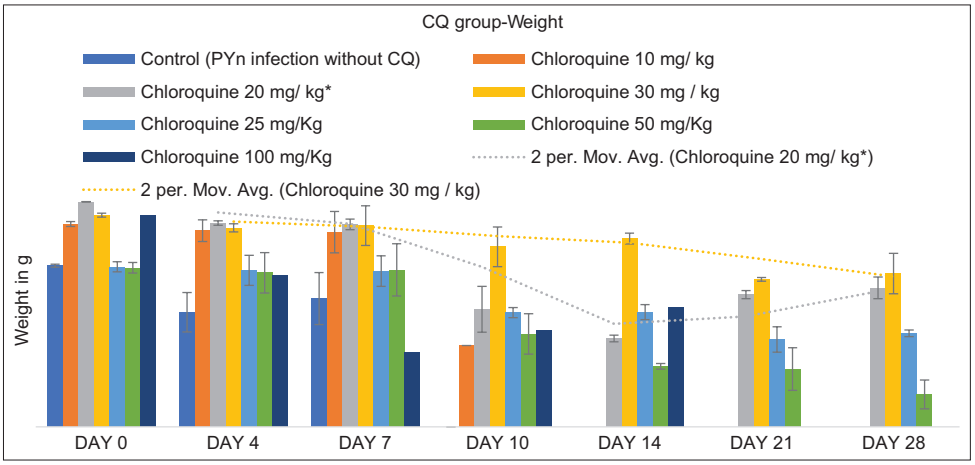
means polar and non-polar phyto-constituents in the extracts of HA and ethanolic both are some phytoconstituent which is not only active for hepato-protective action; even, able to reverse the resistance for CQ or other resistant drugs on PYn.

Nevertheless, all the extracts are effective, HA extract received response at lower and higher doses produced the best possible effect either as potentiating or reversing the resistance when administered along with CQ.

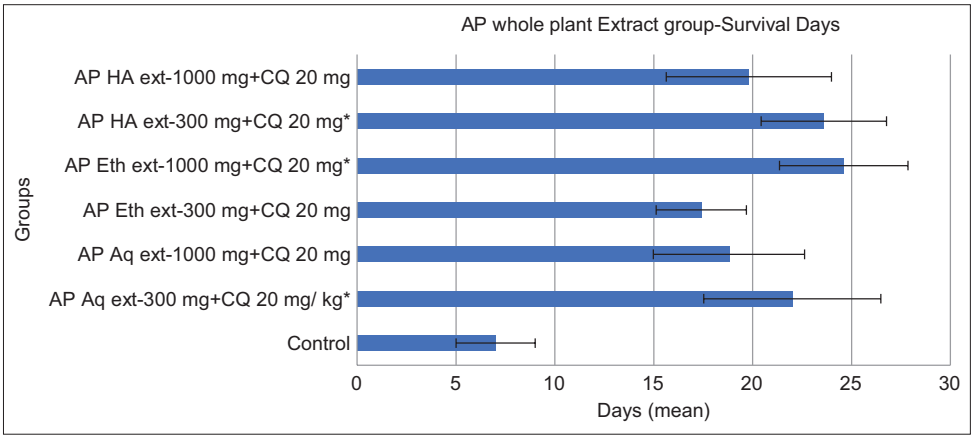
Study of AP whole plant extracts with CQ are hopeful to find some effective phyto-constituent from herbal space which will be an answer for resistance reversal in future. Our objective of research was to assess the possibility of Resistance reversal.



Graph 4: Hemoglobin % optical density comparison chloroquine group

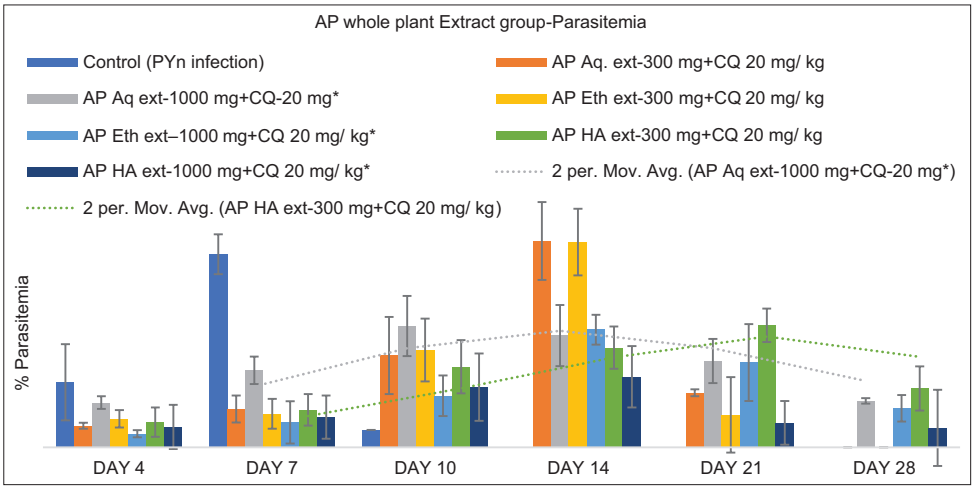


Graph 5: Weight comparison of chloroquine group

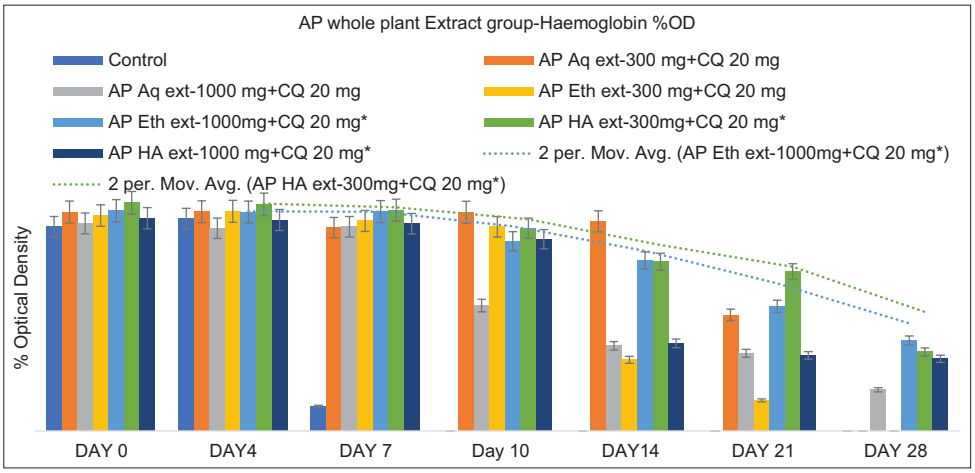


Graph 6: *Andrographis paniculata* Whole plant extract group – survival days

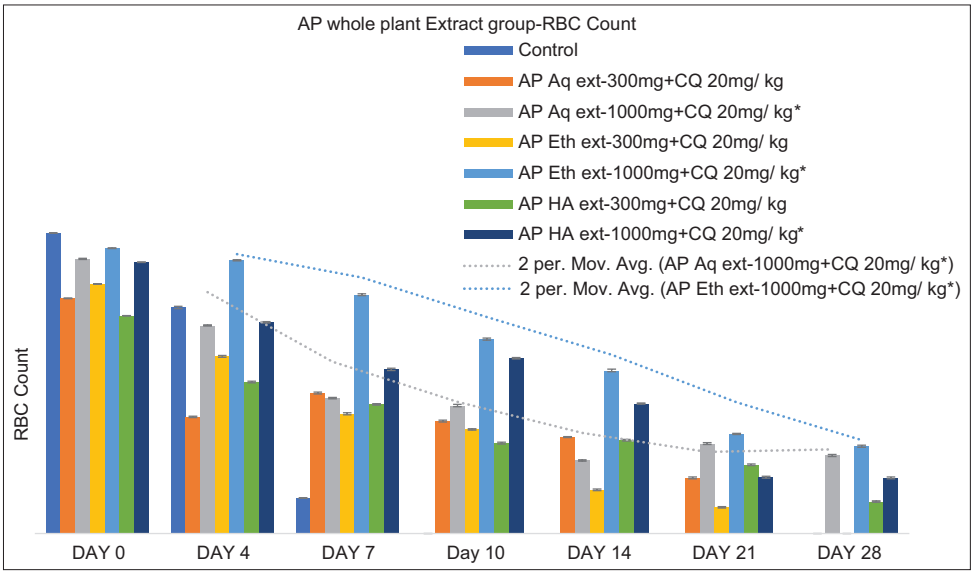
Hence, a molecule can produce the same effect (or better effect) by just making some modification in the adjuvants or co-drugs. Resistance is a global problem now for all sorts of Drugs molecule. Bacteria/plasmodium or recipient develop the immunity, tolerance, resistance on day by day basis. All molecules including newer Artemisinin like medicines reported for mutagenesis even in India. PYn is a proven and resistant plasmodium model (like *P. vivax* for human) to assess the pharmacological and medicinal impacts in Animals. Exactly without studying the Mechanism of Action (MOA) of herbal drug used which is a very difficult task as



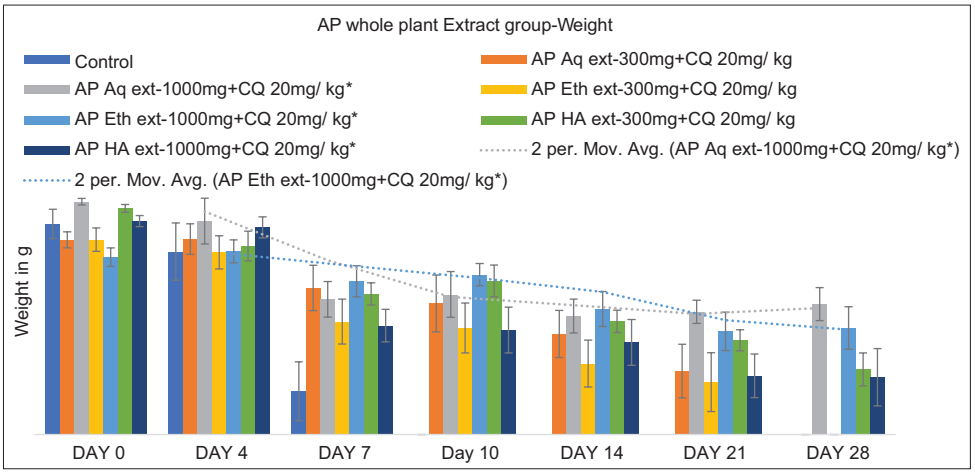
Graph 7: *Andrographis paniculata* whole plant extract Group – Parasitemia



Graph 8: *Andrographis paniculata* whole plant extract group – hemoglobin % Optical Density



Graph 9: *Andrographis paniculata* whole plant extract group – red blood cell count



Graph 10: *Andrographis paniculata* whole plant extract group – weight

Table 1: Most recommended plants during the short survey

Name of the Plant	Botanical Family	Common Name (India)	Part used in Malaria
Ocimum sanctum	Lamiaceae	Tulsi	Leaves
*Andrographis paniculata**	Acanthaceae	Kalmegh	Whole part
Cinchona officinalis	Rubiaceae	Cinchona	Bark
Myrtus communis	Myrtaceae	Vilayti Mehandi	Essential oils
Lantana camara	Verbenaceae	Lentane	All parts
*Azadirachta indica**	Maliaceae	Neem	Leaves and bark
Swertia chirayita	Gentianaceae	Chirata	All parts

Table 2: Detail of plant used for experiments

Name of the Plant	Family	Common Name	Part used in Malaria
*Andrographis paniculata**	Acanthaceae	Kalmegh	Whole part

Table 3: Percent yield of plant extracts

Plant name	Trivial name	Parts used	Extract (s)	% yield
Andrographis paniculate	Kalmegh	Whole part	Aqueous	6.50
			Hydroalcoholic	4.60
			Ethanol	6.20

most of the drug action pathway with pharmacodynamics is still unknown for antimalarial drugs. We cannot prove that the effect of drug in combination is because of potentiating action of drug or a reversal of resistance. Potentiation can be possible by number of ways in the transduction and we don't know exactly what in our extract or where our drugs possess the effect. Since our drug possesses possible reversal of resistance, this made us to assume that any one of the phyto-constituent present in the extract must have good Antimalarial and resistance reversal activity.

Future prospects, the future of antimalarial drug resistance and efforts to combat, it is defined by a number of assumptions. Among other important tasks in global strategy of WHO is to reduce

Table 4: List of material used and their Source

Material	Source/supplier or make
Plant extracts	Shelf collection and processing
Chloroquine sulfate	Gift sample from M/S Tirupati Medicare ltd., Paonta Sahib HP
Carboxy Methyl Cellulose	Gift sample from Tirupati Medicare Ltd.
Giemsa Stain	Available in the lab. Supplier West India Chemicals
Drabkins reagent	Available in lab. Supplier Sigma-aldrich.
TCA buffer	Available in lab. Prepared in lab Trichloroacetic Acid
Camera enabled	Available in lab. Leica Microsystems ltd
Leica microscope	camera – LIECA DFC 320
Spectrophotometer	Available in lab. 96 well plate method.
Filter paper	Whatman

Table 5: Survival days- chloroquine group

Groups	Days (mean)	SD
Control (PYn infection without CQ)	8.14	4.83
Chloroquine 10 mg/kg	11.201	4.65
Chloroquine 20 mg/kg	28.67	3.098
Chloroquine 25 mg/kg	26.87	4.209
Chloroquine 30 mg/kg	23.09	3.903
Chloroquine 50 mg/kg	25.67	4.819
Chloroquine 100 mg/kg	19.45	2.657

*Dose level showing significant activity $P \leq 0.05$

plasmodium resistance. In general, focus on (1) reducing overall drug pressure through more selective use of drugs; improving the way drugs are used through (2) improving prescribing, follow-up practices, and patient compliance; or using drugs or (3) drug combinations which are inherently less likely to foster resistance or have properties that do not facilitate development or spread of resistant parasites.

We consider our finding to re-evaluate with better scientific approach and add resistance reversal with the third global approach of the WHO. Drug combinations are cost-effective in reducing the drugs resistance. First, artemisinin compounds used in combination with a longer acting antimalarial can rapidly reduce parasite densities to very low levels at a time when drug levels of the longer acting antimalarial drug are still maximal. This greatly reduces both the likelihood of parasites surviving initial treatment and the

Table 6: Parasitemia comparison of CQ Group

Group	Day 4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control (PYn Infection without CQ)	32.54	1.98	36	5	0	0	0	0	0	0	0	0
Chloroquine 10 mg/kg	16.76	0.931	24	5	26.87	3.809	0	0	0	0	0	0
Chloroquine 20 mg/kg	9.76	2.793	14.55	2.093	10.2	2.873	15.77	3.548	9.06	1.98	8.9	2.873
Chloroquine 25 mg/kg	10.65	0.365	12.89	2.843	8.81	4.472	20.22	0.435	14.66	0.652	10.9	0.43
Chloroquine 30 mg/kg	8.93	3.786	16.23	0.547	14.54	3.363	18.3	2.567	16.7	0.562	12.05	4.729
Chloroquine 50 mg/kg	16.87	4.437	28.03	3.76	20.65	0.623	22.3	2.658	26.3	4.342	4.6	1.084
Chloroquine 100 mg/kg	12.56	0.879	48.01	2.839	22.65	3.193	45	0.876	0	0	0	0

*Dose level showing significant activity $P \leq 0.05$

Table 7: RBC count of CQ Group

Group	Day 0	SD	Day 4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control (PYn Infection without chloroquine)	1221.2	0.243	1224.08	2.769	918.8	4.61	0	0	0	0	0	0	0	0
Chloroquine 10 mg/kg	1159.6	0.012	1106.34	1.083	780.8	3.095	465.78	4.907	0	0	0	0	0	0
Chloroquine 20 mg/kg*	1044	0.402	1056.98	0.561	815.8	2.657	981.23	2.928	111.2	3.037	193	2.893	118.6	0.249
Chloroquine 25 mg/kg	841.4	0.193	902.43	1.659	741.4	3.928	607.82	3.289	656.4	4.183	242	3.675	436.2	3.792
Chloroquine 30 mg/kg	1155.4	0.108	986.7	2.902	935.2	2.793	569.09	2.659	637.8	2.874	543.6	3.872	564.6	2.615
Chloroquine 50 mg/kg	1064.2	0.207	1098.89	1.762	735	3.017	702.35	2.703	681.6	3.872	463	0.023	162	4.658
Chloroquine 100 mg/kg	1052.8	0.365	923.56	2.805	865.2	2.472	543.7	4.182	675.4	3.982	0	0	0	0

*dose level showing significant activity $P \leq 0.05$

Table 8: Hemoglobin % optical density comparison of CQ groups

Groups	Day 0	SD	Day 4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control (PYn Infection without CQ)	0.2412	0.032	0.2031	2.892	0.18	4.763	0	0	0	0	0	0	0	0
Chloroquine 10 mg/kg	0.2404	0.176	0.2501	3.276	0.1446	1.092	0.0798	4.093	0	0	0	0	0	0
Chloroquine 20 mg/kg*	0.2232	0.276	0.2091	0.437	0.224	0.902	0.0985	4.08	0.196	2.02	0.22	0.345	0.3001	1.099
Chloroquine 25 mg/Kg	0.2096	0.269	0.2134	2.65	0.208	2.093	0.1592	1.87	0.1612	4.65	0.139	0.692	0.1178	2.09
Chloroquine 30 mg/kg	0.2458	0.832	0.2035	4.769	0.1632	3.769	0.1108	2.873	0.1206	2.902	0.113	2.05	0.1406	0.43
Chloroquine 50 mg/Kg	0.1796	1.008	0.1876	3.78	0.224	2.043	0.0981	4.048	0.1534	3.097	0.0606	5	0.0396	0.298
Chloroquine 100 mg/Kg	0.1926	0.378	0.2001	2.02	0.2304	3.65	0.0892	4.384	0.169	4.183	0	0	0	0

Dose level showing significant activity $P \leq 0.05$

Table 9: Weight comparison of CQ group

Group	Day 0	SD	Day 4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control (PYn infection without CQ)	30.9375	0.283	22.01	3.782	24.61	5	0	0	0	0	0	0	0	0
Chloroquine 10 mg/kg	38.9	0.458	37.62	2.084	37.34	3.98	15.63	0	0	0	0	0	0	0
Chloroquine 20 mg/kg*	43.168	0.02	39.078	0.43	38.828	1.009	22.54	4.392	16.976	0.639	25.362	0.769	26.658	2.08
Chloroquine 30 mg/kg	40.59	0.376	38.17	0.761	38.59	3.817	34.54	3.793	36.11	1.023	28.3	0.371	29.424	3.879
Chloroquine 25 mg/Kg	30.7	0.986	30.022	2.879	29.88	2.932	21.92	0.981	22.01	1.457	16.766	2.438	17.95	0.658
Chloroquine 50 mg/Kg	30.502	1.02	29.544	3.872	30.13	5	17.85	3.873	11.616	0.547	11.106	4.084	6.246	2.76
Chloroquine 100 mg/Kg	40.5	0.08	29	4.692	14.28	3.576	18.54	2.068	23	0.356	0	0	0	0

*Dose level showing significant activity $P \leq 0.05$

Table 10: AP whole plant group - survival days

Group	*Days (mean)*	*SD*
Control	7	2
AP Aq ext-300 mg + CQ 20 mg/kg*	22	4.477
AP Aq ext-1000 mg + CQ 20 mg	18.8	3.826
AP Eth ext-300 mg + CQ 20 mg	17.4	2.279
AP Eth ext-1000 mg + CQ 20 mg*	24.6	3.25
AP HA ext-300 mg + CQ 20 mg*	23.6	3.172
AP HA ext-1000 mg + CQ 20 mg	19.8	4.176

*Dose level showing significant activity $P \leq 0.05$. AP: *Andrographis paniculata*, CQ: Chloroquine

likelihood that parasites will be exposed to suboptimal levels of the longer acting drug.[22] In our findings where resistance reversal looks possible with herbal extracts can provide less time to parasite for exposure at sub-optimal levels. Secondly, multiple mechanism of action of one phyto-constitute (which are pharmacological active moiety, multiple active moieties are possibly the principle behind either to delay resistance or possible resistance reversal.

After the due trials and research, we propose following work still need to be done to apply the above delay in resistance or possible resistance reversal.

Refinement of Dose for CQ and AP for Better and Accurate Anti-malarial Pharmacological Action and Possible Resistance Reversal

Refinement of doses for AP HA (300 mg/kg or more), Ethanolic extract 1000 mg/kg or less was promising the antimalarial effect

Table 11: AP Whole plant extract group – parasitemia

Group	Day 4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control (PYn infection)	8.142	4.761	24.176	2.491	2.166	0	0	0	0	0	0	0
AP Aq. ext-300 mg+CQ 20 mg/kg	2.7	0.384	4.8	1.679	11.5	4.826	25.82	4.871	6.82	0.438	0	0
AP Aq ext-1000 mg+CQ-20 mg*	5.6	0.792	9.64	1.729	15.18	3.762	13.98	3.832	10.806	2.769	5.8	0.345
AP Eth ext-300 mg+CQ 20 mg/kg	3.564	1.093	4.2	1.873	12.18	3.917	25.7	4.173	4.06	4.719	0	0
AP Eth ext–1000 mg+CQ 20 mg/kg*	1.706	0.438	3.12	2.652	6.454	2.546	14.724	1.873	10.616	4.81	4.88	1.657
AP HA ext-300 mg+CQ 20 mg/kg	3.16	1.84	4.692	1.983	10.08	3.329	12.472	2.643	15.274	2.081	7.36	2.765
AP HA ext-1000 mg+CQ 20 mg/kg*	2.546	2.768	3.78	2.693	7.538	4.21	8.84	3.839	3.06	2.739	2.44	4.765

Dose level showing significant activity $P \leq 0.05$. AP: *Andrographis paniculata,* CQ: Chloroquine

Table 12: AP whole plant extract group – Haemoglobin % OD

Group	Day 0	SD	Day4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control	0.3087	0.234	0.3206	1.43	0.03707	3.659	0	0	0	0	0	0	0	0
AP Aq ext-300 mg+CQ 20 mg	0.3308	0.546	0.3314	1.094	0.3073	2.926	0.3305	2.651	0.3167	3.905	0.1753	4.982	0	0
AP Aq ext-1000 mg+CQ 20 mg	0.3138	0.454	0.3063	0.456	0.3086	1.874	0.1894	3.025	0.1286	3.815	0.1174	3.24	0.06224	3.451
AP Eth ext-300 mg+CQ 20 mg	0.3255	0.121	0.3318	2.345	0.3175	2.592	0.3086	3.017	0.1074	4.056	0.04614	3.564	0	0
AP Eth ext-1000mg+CQ 20 mg*	0.3328	0.343	0.3309	1.239	0.3317	3.902	0.2867	4.901	0.2579	3.762	0.1883	2.145	0.1368	2.256
AP HA ext-300mg+CQ 20 mg*	0.3448	0.343	0.3424	0.437	0.3336	3.038	0.3065	2.879	0.2559	4.816	0.2406	4.32	0.1195	3.567
AP HA ext-1000 mg+CQ 20 mg*	0.3216	0.165	0.3189	0.879	0.3132	2.802	0.2898	3.601	0.1324	3.706	0.1141	4.643	0.1091	4.8779

Dose level showing significant activity $P \leq 0.05$. OD: Optical density, AP: *Andrographis paniculata,* CQ: Chloroquine

Table 13: AP whole plant extract group – RBC count

Group	Day 0	SD	Day 4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control	1221.2	0.124	918.8	4.839	144	0.456	0	0	0	0	0	0	0	0
AP Aq ext-300 mg+CQ 20 mg/kg	956.6	1.345	474.2	2.657	571.4	3.573	456.78	3.692	391.8	2.095	225.76	3.821	0	0
AP Aq ext-1000 mg+CQ 20 mg/kg*	1116.2	2.324	846	2.768	550.2	2.769	520.12	4.673	297	2.723	365.76	3.987	317.4	4.287
AP Eth ext-300 mg+CQ 20 mg/kg	1015.2	0.456	720	3.978	486.4	4.327	422.76	2.734	177.6	3.657	106.5	2.546	0	0
AP Eth ext-1000 mg+CQ 20 mg/kg*	1160.4	1.453	1111	2.692	971.2	3.762	790.32	4.21	663	4.923	404.56	2.545	354.6	4.237
AP HA ext-300 mg+CQ 20 mg/kg	885.6	0.556	616	3.092	525	2.657	367.76	3.692	379.4	3.376	278.98	3.212	130	2.767
AP HA ext-1000 mg+CQ 20 mg/kg*	1103	0.879	858.8	2.438	667.2	4.729	712.86	2.672	528	2.557	229.09	3.765	226	3.876

Dose level showing significant activity $P \leq 0.05$, AP: *Andrographis paniculata,* CQ: Chloroquine, RBC: Red blood cell

Table 14: AP whole plant extract group – weight comparison

Group	Day 0	SD	Day 4	SD	Day 7	SD	Day 10	SD	Day 14	SD	Day 21	SD	Day 28	SD
Control	35.2	2.453	30.56	4.792	7.21	4.917	0	0	0	0	0	0	0	0
AP Aq ext-300 mg + CQ 20 mg/kg	32.54	1.342	32.65	2.564	24.5	3.792	21.9	4.762	16.8	3.927	10.6	4.498	0	0
AP Aq ext-1000 mg + CQ 20mg/kg*	38.92	0.543	35.67	3.829	22.6	2.982	23.4	3.832	19.8	2.845	20.5	1.935	21.8	2.76
AP Eth ext-300 mg + CQ 20 mg/kg	32.56	1.954	30.45	2.768	18.87	3.76	17.8	4.173	11.86	3.917	8.76	4.916	0	0
AP Eth ext-1000 mg + CQ 20 mg/kg*	29.65	1.543	30.6	1.925	25.6	2.619	26.7	1.873	20.98	2.925	17.23	3.218	17.8	3.554
AP HA ext-300 mg + CQ 20 mg/kg	37.8	0.654	31.5	2.492	23.5	1.863	25.67	2.643	18.9	1.872	15.76	1.769	10.87	2.765
AP HA ext-1000 mg + CQ 20 mg/kg*	35.67	0.927	34.61	1.769	18.21	2.715	17.45	3.839	15.4	3.827	9.8	3.658	9.56	4.786

Dose level showing significant activity $P \leq 0.05$. AP: *Andrographis paniculata,* CQ: Chloroquine

along with CQ. Refinement and better adjustment of doses of when combining CQ 20 mg/kg for resistant plasmodium. A promising approach is needed to use these agents as templates for resistance reversal. Search for additional antimalarials their combinations from higher plants must continue to fight the one of the deadliest diseases on earth.

Stage Selective Screening of AI Extracts

To know mechanism of its pharmacological action and possible resistance reversal. These options must be with more plant extracts. Or using respective phyto-constituents/phytomarkers.

Resistance Reversal in Other Pharmacological Area Where Resistance Predominant

*E*xplore the same concept of using the promising plant extracts along with synthetic drugs for the diseases where resistance is a threat. For example, leprosy, antimicrobial.

Overall, *in vivo* antimalarial activity can be classified as "moderate", "good", and "very good" based on the response of plant extract. A percentage parasitemia suppression ≥50% at a dose of 500 mg/kg, 250 mg/kg, and 100 mg/kg body weight/day, respectively.[23] According to this classification, the AP whole plant extracts have Moderate anti-plasmodial activity.

Conclusions

The finding of study revealed that HA extracts and ethanolic extract of AP whole plant are able to produce antimalarial effect on CQ resistant PYn when given along with CQ. Which are mainly due to their activity on parasitemia probably due to their efficiency on hepatic cells. Even though, all plant extracts are insignificant when given alone without CQ. Overall, blood health, RBC count, and Hb do not resurrected significantly. However, survival time of the extract-treated mice was also significantly prolonged. In the study, both extracts; HA extract of leaves and ethanolic extract of bark produced significant parasitemia suppression in early days. Same while, both the extracts are able to reverse the either of schizontocidal activity of CQ or potentiate the CQ activity. Specific and more studies are required to determine the phytoconstituent because of whom resistance reversal or potentiation CQ activity was possible in our activity. Further to phyto-constituent activity, parasite stage selective activity, mechanism of action of reversal and dose differentiating activities of extracts also needs to explore. Therefore, objective of this study cannot include all multitudes of factors, but slight positive results may pose an affirmative hope and pave a path for inclusion of herbal extracts or herbs as side treatment or resistance-breaker food for malaria along with existing treatment of malaria. Finding of this study are significantly able to postulate theory of resistant food for malaria or adjuvant therapy for malaria alongside of main treatment to reduce the resistance burden on single therapeutic molecule.

References

1. World Health Organization. World malaria Report. Geneva: World Health Organization; 2020.
2. Wright WC. Plant derived antimalarial agents, new leads and challenges. Phytochem Rev 2005;4:55-61.
3. Batista R, de Jesus Silva A Jr., Oliveira BA. Plant-derived antimalarial agents, new leads and efficient phytomedicines, part II. Non-alkaloidal natural products. Molecules 2009;14:3037-72.
4. Beckmann H. In Antimalarial Drugs: Their Nature, Action and Use. Geneva: World Health Organization 2001. p. 529-33.
5. Bharel S, Gulati A, Abdin MZ, Srivastava PS, Jain SK. Structure, biosynthesis and function of Artimisin. Fitoterapia 1996;67:387-99.
6. Mukherjee T. Antimalarial herbal drugs. A review. Fitoterapia 1991;62:197-204.
7. Sharma P, Sharma JD. Plants showing antiplasmodium activity from crude extracts to isolated compounds. Indian J Malariol 1998;35:57-110.
8. Klayman DL. Quinghaosu (Artemisinin); an antimalarial drug from China. Science 1985;228:1049-55.
9. Bloland PB. Drug resistance in Malaria. Geneva: World Health Organization, Background Documents for Who Global Strategy; 2001.
10. Foster SD. Pricing, distribution, and use of antimalarial drugs. Bull World Health Organ 1991;69:349-63.
11. Ridley RG. Plasmodium: Drug discovery and development-an industrial perspective. Exp Parasitol 1997;87:293-304.
12. Bruce-Chwatt LJ, Black HR. Chemotherapy of Malaria. Geneva: World Health Organization, 1986.
13. Lobel HO, Campbell CC. Malaria Prophylaxis and distribution of drug resistance. Clin Trop Med Communicable Dis 1986;1:225-42.
14. Tripathi KD. Essentials of Medical Pharmacology. 6th ed. India: Jaypee Brothers Medical Publishers; 2013. p. 785.
15. Bray PG, Boulter MK, Ritchie GY, Howell RE, Ward SA. Mol Biochem Parasitol 1994;63:87-94.
16. Bray PG, Boulter MK, Ritchie GY, Howell RE, Ward SA. Mol Biochem Parasitol 1994;44:1317-24.
17. Nagalekshmi R, Menon A, Dhanya KC, Nair CK. Hepatoprotective activity of *Andrographis paniculata* and *Swertia chirayita*. Food Chem Toxicol 2011;49:3367-73.
18. Widyawaruyanti A, Asrory M, Ekasari W, Setiawan D, Radjaram A, Tumewu L, *et al*. *In vivo* antimalarial activity of *Andrographis paniculata* Tablets. Proc Chem 2014;13:101-4.
19. Peter W, Portus H, Robinson L. The four-day suppressive *in vivo* antimalarial test. Ann Trop Med Parasitol 1995;69:155-71.
20. Ryley JF, Peters W. The antimalarial activity of some quinoline esters. Ann Trop Med Parasitol 1995;84:209-22.
21. Kalra BS, Chawla S, Gupta P, Valecha N. Screening of antimalarial drugs. Ind J Pharmacol 2006;38:5-12.
22. White NJ, Nosten F, Looareesuwan S, Watkins WM, Marsh K, Snow RW, *et al*. Averting a malaria disaster. Lancet 1999;353:1965-7.
23. Munoz V, Sauvain M, Bourdy G. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. J Ethnopharmacol 2000;69:127-37.