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Effectiveness of Artesunate and Mefloquine combination therapy in the treatment of uncomplicated *Plasmodium falciparum* Malaria in Eastern India: A cross sectional study

Roy Sasmit [1] , Maitra Somnath[2], Guha S.K[3] Maji A.K[4], Raychauduri Samik[5]
Corresponding Author; Maitra Somnath

Abstract
BACKGROUND-Plasmodium falciparum(Pf) malaria is a major public health problem in India.Unfortunately, Pf has developed resistance to major antimalarial drugs,so malaria treatment moved in the era of Artemisinin Combination Therapy.
AIMS -
To evaluate the efficacy and tolerability of Artesunate and Mefloquine (AS+MQ) combination in uncomplicated Pf malaria in Kolkata,West Bengal.
MATERIALS AND METHODS-
The cross-sectional study was performed in the Malaria Clinic, School Of Tropical Medicine, Kolkata with 104 patients above 6 months of age.After a rapid screening procedure, patients fulfilling the inclusion criteria took part in the study. Peripheral blood smear,HRP2 Strip test were done for diagnosis. The participants were randomized to receive AS+ MQ. The study was done according to W.H.O PROTOCOL 2003 and appropriate follow up was performed.
RESULTS-
Data showed that mean fever clearance time was 2 days after initiating the drugs and mean parasite count also became zero by day2.Paired samples correlation of temperature was also significant. Kaplan Meier analysis showed that there was no treatment failure in the study arm.
CONCLUSION-
AS+MQ combination therapy is highly effective in the treatment of uncomplicated Pf malaria and the combination is also safe and tolerable.
Keywords- *Plasmodium falciparum; Artesunate and mefloquine.*

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I. Introduction

Malaria, a protozoan disease transmitted by the bite of infected female anopheles mosquitoes, infects approximately 5% of the world's population and causes 1 to 3 million deaths per year. Plasmodium falciparum(Pf) predominates in Africa,New Guinea and Haiti while Plasmodium vivax (Pv) is prevalent in Central America.The prevalence of these two species is approximately equal in South America ,India,Eastern Asia& Oceania.[1,2]

Malaria is one of the major public health problems in India.Recently,1.49 million cases of malaria(including 0.77 million Pf cases) and 767 deaths were reported in 2010.(Provisional data given by National Vector Borne Disease[NVBD]).The reported Pf cases declined from 1.14 million in 1995 to 0.77 million cases in 2010. However, the percentage of Pf cases gradually increased from 39% to 52.12% in 2010.[3]

Unfortunately, Pf has developed resistance to nearly all classes of available antimalarial drugs, leading to resurgence of malaria morbidity and mortality in most endemic areas[4,5].

In India chloroquine(Cq)resistant Pf was first reported from Diphu areas of Karbi,Analog district of Assam in 1973,& then it spreads to various parts of the country[6,7].Accordingly,Indian Drug Policy was changed to a combination of S.P(Sulphadoxine-Pyrimethamine) as a second line of treatment in 1982 [8]. However, a low level of in vivo resistance to this drug combination has been reported from various parts of India[9,10,11].

Studies on molecular markers related with SP therapy showed a progressive increase in mutations associated with this [12].Due to emergence of resistance, the malaria treatment moved into the era of Artemisinin Combination Therapy(ACT).ACTs were designed to attack the parasites with 2 or more drugs with different mechanisms of action reducing the probability emergence of resistance [13].

AIM
To evaluate the efficacy & tolerability of Artesunate and Mefloquine combination in uncomplicated Pf malaria in Kolkata, West Bengal.

II. Materials And Methods

STUDY SITE
Malaria Clinic, School of Tropical Medicine (STM) ,Kolkata.

STUDY POPULATION
Patients of both genders above 6 months of age.

FOLLOW UP
Days 0, 1, 2, 3, 7, 14, 21 and 28

SCREENING EVALUATION
A rapid screening procedure was done in an outpatient setting to identify patients who met the enrolment criteria. The screening data included age, gender, body temperature, body weight and height. Thick and thin peripheral blood smear (PBS) was examined.

ENROLLMENT
All patients with confirmed *Pf* infection, above 6 months and fulfilling inclusion criteria was explained about the benefits and risks of the study. Written, informed consent was taken.

PARAMETERS STUDIED
i) History
ii) Measurement of axillary temperature
iii) Laboratory examinations at scheduled follow-up

INCLUSION CRITERIA
• Age-> 6 months
• Mono infection with P.falciparum
• Parasitaemia in the range of 1000 -100000/μL
• Axillary temperature of $\geq 37.5^0$C or history of fever in previous 24 hours
• Informed consent of the patient/guardian.

EXCLUSION CRITERIA
• Presence of one or more of the general danger signs or any sign of severe or complicated malaria.
• Presence of mixed infection.
• Presence of febrile conditions other than malaria.
• Presence of severe disease.
• Contraindication to anti malarials.
• Pregnancy[14].

SAMPLE SIZE
104 patients was enrolled

LABORATORY EXAMINATION
PBS was prepared for all subjects, stained by Giemsa stain and examined under microscope for parasite count.
HRP 2 Strip test
Pregnancy test, wherever indicated

TREATMENT
The participants were randomized to receive Artesunate + Mefloquine.
Dosing schedule of Artesunate and Mefloquine as per WHO guideline (2006)
Artesunate (AS): each tablet containing **50 mg**; **Mefloquine (MQ)**: each tablet containing **250 mg.**
The drugs were procured from Malaria Clinic, School of STM, Kolkata. (Primaquine, as per NVBDCP schedule, was also administered)

Age	Drug	1st day (no. of tablets)	2nd day (no. of tablets)	3rd day (no of tablets)
<1	AS MQ	½ nil	½ ½	½ Nil
1-6	AS MQ	1 nil	1 1	1 Nil
7-13	AS MQ	2 nil	2 2	2 1
>13	AS MQ	4 nil	4 4	4 2

Primaquine for P. falciparum (Single dose on Day 2)[4]

Age in years	Dosage (in mg base)	No. of tablets (7.5 mg base)
<1	Nil	0
1-4	7.5	1
5-8	15	2
9-14	30	4
15 & above	45	6

Provision of alternative anti malarial drugs for treatment failure cases
Quinine (tablet & injection), Artesunate injection, Doxycycline tablet was kept in reserve for treatment of severe and complicated malaria and for 'treatment failure' cases.

Study Design –WHO protocol 2003:
This protocol consists of recording essential patient information, clinical assessment, axillary temperature, parasitaemia, bodyweight on day 0 (prior to treatment) with the stipulated drug, clinical assessment with examination of axillary temperature on days 1,2,3,7,14,21 & 28 and parasitological examination on days 2,3,7,14,21 & 28.

• On day 1 the patient was examined for parasitaemia and any danger sign.

• On day 2 or any other day the patient was also examined for parasitaemia, and danger sign or clinical deterioration.

Classification of therapeutic response according to WHO protocol 2003:
There are three categories of therapeutic responses, namely 'Early Treatment Failure'[15] (ETF), 'Late Treatment Failure' (LTF) and 'Adequate Clinical and Parasitological response (ACPR)'.

PARAMETERS STUDIED:
As per WHO protocol-2003, clinical assessment, measurement of axillary temperature and parasitological examinations of the patients was done on Day 0,2,3,7,14,21,28 of follow up due to non-compliance of patients to attend clinic daily seven days.

Clinical Assessment
A pre treatment clinical examination including general survey and systemic examination were done in each patient. Besides, body weight was also recorded . All the above assessments were repeated on day 1, 2, 3, 7, 14, 21and 28.

STUDY TOOLS AND TECHNIQUES:
Laboratory Diagnosis
PBS of all patients were examined on day 0, 2, 3, 7, 14, 21, 28 and on any unscheduled day.
Collection of Blood and blood slide Preparation
Both thick and thin smears were taken in same slide from finger pricked blood sample.

Staining
Before staining, thick blood smears were dehaemoglobinised with distilled water, dried (both thick and thin) and fixed with acetone free methanol for 2 minutes. The fixed smears were stained with diluted stock Giemsa solution with buffer water at the ratio of 1:3 for 10 minutes.

Stained slides were washed by over flooding them with tap water and examined them under oil immersion lens after drying.

Slide: Giemsa stained thin blood smear showing *P.falciparum* ring parasite load was measured by counting number of asexual forms of *Plasmodium falciparum* parasites against 200 leucocytes present in stained thick blood smear. In case of low parasitaemia (<10/200 leucocytes), counting was done against 500 leucocytes. The parasite load was calculated by applying the following formula:

Parasitaemia (per micro litre) = number of parasites X 8000 / number of leucocytes

A blood slide was declared negative when examination of 100 thick smear fields did not show the asexual forms of *Plasmodium falciparum*.

STATISTICAL METHODS
In the following study apart from mean,standard deviations. Kaplan-Meier, paired t test and correlations were also used.

III. Results And Analysis

The present study was started in the Malaria Clinic of STM, Kolkata. 104 parasitologically confirmed and antigen positive *Plasmodium falciparum* patients were recruited. All the enrolled patients were administered Artesunate + Mefloquine (AS+MQ) tablets as per study protocol after obtaining due consent. The patients were thoroughly explained about the method of taking drugs and follow up schedule, informed about possible adverse effects and requested to report immediately in case of any adverse reactions occuring relating to the disease during the follow up period. 14 patients were eventually lost to follow up during the subsequent scheduled days.

The study showed the following data:

Age and gender
Of the 104 recruited, 78 turned out to be male (78/104; 75%) while the rest(26/104; 25%) were females. Mean age of presentation was 29.4 years (Sd +12.21, range 7-60 yrs.)

Table 1: Age distribution

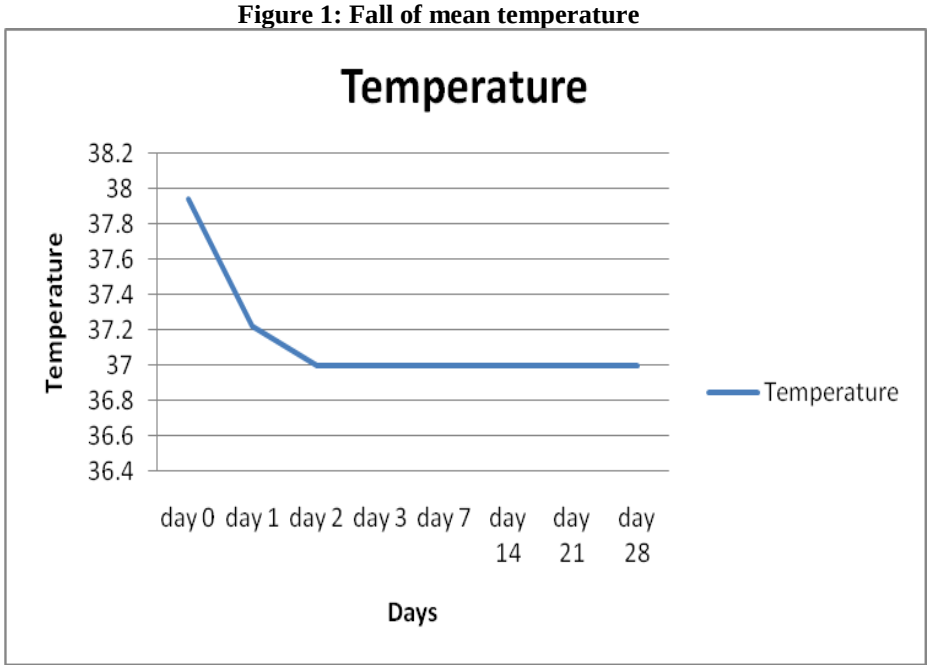
Variables (Age in years)	Study population
Mean	29.47
Range	7-60
Standard deviation (SD)	+12.21

Temperature
Pf parasitologically confirmed patients with fever of >37.5°C or with history of fever within the previous 24 hours were enrolled. On day 0, the mean temperature in the AS+MQ group was 37.94°C. The details of temperature on Day 0, 1, 2,3,7,14,21 and 28 are shown in Table 2. From Table 2, it is evident that the mean temperature decreased from day 0 to day 2.The dynamics of mean axillary temperature is given in figure 1.

Table 2: Variations in temperature

Day	Variation	Temperature (˚C)
Day 1	Mean SD	37.22 + 0.117
Day 2	Mean SD	37 0
Day 3	Mean SD	37 0
Day 7	Mean SD	37 0
Day 14	Mean SD	37 0
Day 21	Mean SD	37 0
Day 28	Mean SD	37 0

Figure 1: Fall of mean temperature



Fever Clearance Time (FCT)
Data showed that the mean FCT of all patients was 2 days after initiating the drugs.

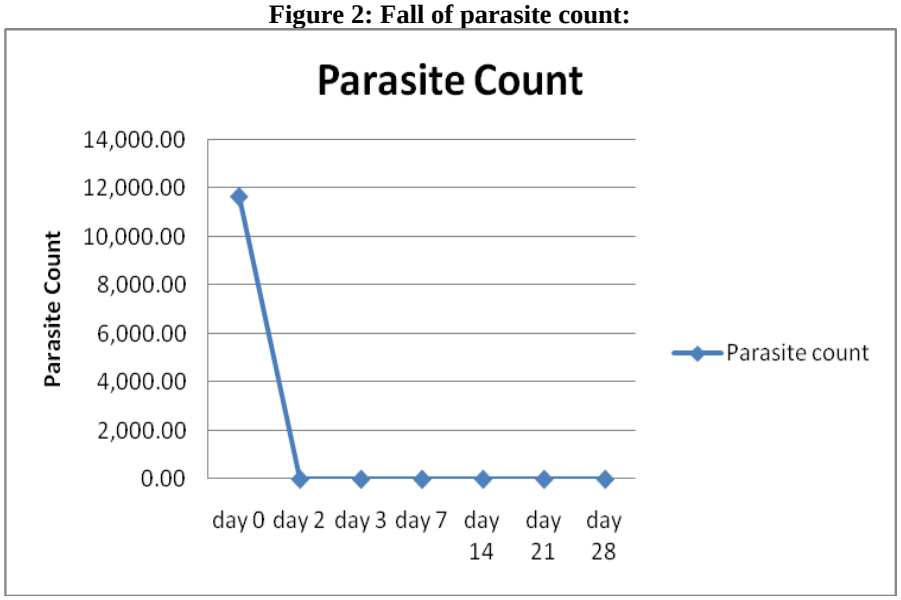
Parasite Count
Patients with at least one *Plasmodium falciparum* parasite for every 6-8 WBCs corresponding to approximately 1,000 asexual parasites per micro Litre were enrolled for the study. On day 0, the mean parasite count was 11,645.09 /µL (range 1,000 - 91,000). The details of parasite count on Day 0, 2,3,7,14,21 and 28 are depicted in Table 3

Table 3: Parasite count

Day	Variation	Parasite count /Ml
Day 0	Mean Range SD	11,645.09 1,000 - 91,000 +17,152.15
Day 2	Mean Range SD	0 0 0
Day 3	Mean Range SD	0 0 0
Day 7	Mean Range SD	0 0 0
Day 14	Mean Range SD	0 0 0
	Mean Range SD	0 0 0
Day 28	Mean Range SD	0 0 0

Parasite Clearance Time (PCT):
Figure 2 showed that the mean parasite count became zero by Day 2.

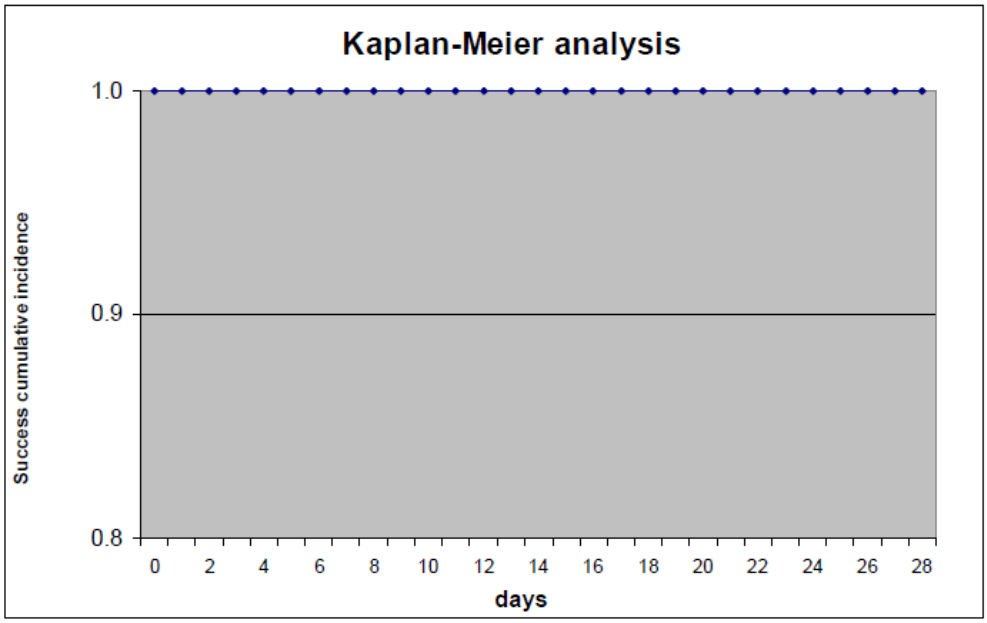
Figure 2: Fall of parasite count:



Outcome

Out of the 104 patients enlisted, total 90 patients (86.5%) showed Adequate Clinical and Parasitological Response (ACPR) while the remaining 14 could not be traced anyhow as they were lost to follow up (LFU) ultimately (13.5%).

Figure 3: Kaplan-Meier analysis of study group



Kaplan-Meier analysis (Figure 3) showed that there was no treatment failure in the study arm.

Among the adverse effects recorded, 4 patients had vomiting, 3 had insomnia and 5 had dizziness and reeling of head on 2nd day after taking the drugs. They were prescribed anti-emetic, anxiolytic and paracetamol etc. for symptomatic management of recorded side effects and advised to take rest for the days of taking anti malarial drugs. They reported to have cleared the side effects on following our advice. Follow up of these few patients revealed they had all taken the drugs accordingly and reported on the scheduled days with all clearing the parasitaemia by day 2.

A paired sample t-test on the temperature at day 0 and the temperature at day 28 was performed.

Sample Statistics:

	N	Mean	SD
Temperature Day 0	90	37.9122	0.54
Temperature Day 28	90	37.0056	0.05

Paired Samples Correlations:

	N	Correlation	Significance
Temperature Day 0 & Temperature Day 28	90	0.2345	0.026

The above table shows that there is high correlation and statistical significant (P-value = 0.026 < 0.05).

Paired Samples t Test: (Temperature Day 0 – Temperature Day 28) :

Parameter	**Value**
N	90
Mean	0.9067
Sample SD	0.53
Std. Err.(Mean)	0.0559
Deg. of Freedom	89
t Statistic	16.2304
P-value	0.0000

The P-value shows that the probability of the t statistic occurring by chance alone is 0.00. In summary, a significant drop in temperature was observed by using the treatment, which cannot be attributed to chance happening.

IV. Discussion:

The cross-sectional study was conducted to evaluate the efficacy and tolerability of AS+MQ combination in patients of uncomplicated *Pf malaria* in Central Kolkata .

Anti-malarial drug efficacy was assessed as per WHO 2003 protocol. The follow up schedule of this protocol on Day 0, 1, 2,3,7,14,21 and 28 was strictly maintained.

By setting up prior inclusion and exclusion criteria, a total of 104 patients were randomly enrolled of whom 90 could complete the recommended follow up.

Gender inequality was evident from the trial with 78 male (75%) patients studied and only 26 female (25%) landing up in the roster. Female patients resented from the study citing inability to come for regular follow ups for social reasons.

The mean age of all patients (n=104) was 29.4 (range 7-60) years.

The mean Fever Clearance Time (FCT) of all patients was within 48 hours of initiating antimalarial drugs.

On day 0, the mean parasite count was 11,645.09/ μL (range 1,000-91,000).The mean parasite count came down to zero by 48 hrs of drug initiation.

It was observed that 86.5% (90/104) showed APCR and 13.5% (14/104) were lost to follow up. There was no early or late treatment failures recorded.

It was observed that 10% (9/90) developed some adverse effects during the course of taking anti malarial drugs.

It is evident from the study that this ACT regimen (AS+MQ) has high efficacy (ACPR 86.5%) in treating uncomplicated *Plasmodium falciparum* malaria.

Also the drug combination is well tolerated among patients and can be safely given to any patients unless he/she has any of the known contraindications to the prescribed drugs specially Mefloquine.

V. Conclusion:

Artemisinin derivatives are regarded as highly effective antimalarial compounds. They are the fastest acting anti-malarial drugs available today.

To prolong the efficacy of these drugs, it is recommended that they should only be used in combination with another effective drug that has a longer half-life.

From the present study it is evident that the AS+MQ combination is efficacious in treating uncomplicated *Plasmodium falciparum* malaria in Kolkata and it can be considered as a viable alternative. It is to be noted that NVDCP of Government of India is currently using the 3-days Artesunate plus Sulphadoxine-Pyrimethamine in all uncomplicated falciparum malaria cases. The fixed dose co- formulation of Artesunate and Mefloquine is now available in the Indian market,whose cost is however higher .But there is a concern for increasing risk of resistance . Regular surveillance should be carried out to identify the appropriate ACT for our country. In addition, efficacy of other ACT regimens of Artemether with Lumefantrine, Artesunate with Amodiaquine, Dihydroartemisinin with Piperaquine, Doxycycline, Clindamycin etc. should also be explored.

Considering the growing SP resistance, it's important to identify other potent, well-tolerated Artemisinin combination therapy (ACT) for the public health programme. The high efficacy of the combination of Artesunate plus Mefloquine provides one more option of Artemisinin combination therapy (ACT) for treatment of uncomplicated *P. falciparum* malaria in India.

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References

[1]. White J Nicholas, Breman G Joel .Malaria .In: Fauci AS, Braunwald E, Kasper DL Eds. Harrison's Principle of Internal Medicine.17th ed.New York,NY: Mc Graw Hill Medical;2008:1280-1293.
[2]. White J Nicholas.Malaria. In:Cook C Gordon.Manson's Tropical Diseases, 21st ed. Saunders Ltd;2009:1205-1295.
[3]. National Drug Policy on Malaria 2010:Directorate of National Vector Borne Diseases and Control Programme (NVBDCP)2010.(Accessed April 15,2013 at http://nvbdcp.gov.in/malaria3.html .
[4]. Snow RW, Noor M,Hay SL, et al.The global distribution of clinical episodes of P. falciparum malaria.Nature 2005;434:2147
[5]. Bray PG, Martin RE,Tilley L,Word SA,Kiri K,Fidock .Defining the role of pfCRT in p.falciparum Chloroquine resistance. Mol microbial 2005;56:323-333.
[6]. Sharma, V.P.Current scenario of malaria in India.Parasitologia 1999 41:349-353.
[7]. Vinayak S, Biswas S, Deb V, et al. Prevalence of K76t mutation in the Pfcrt gene of P.falciparum among Chloroquine responders in India. Acta Trop 2003 ;87:287-293.
[8]. Mishra S.P. In vivo resistance to Chloroquine and sulphadoxine-pyrimethamine combination in P.falciparum in India. Proc. Natl. Acad. Sci. India 1996 ;66:123-138.
[9]. Ahamed A,Balaria D, Vinayak S, Yameen M, Biswas S,Deb V, et al .(2004). Plasmodium falciparum isolates in India exhibit a progressive increase in mutations associated with sulphadoxine-pyrimethamine resistance. Antimicrob Agents Chemother 2004;48:879-889.
[10]. Ahamed A, Das M K,Deb V,et al.Quadruple mutations in dihydrofolate reductase of P.falciparum isolates from Car Nicobar Island, India. Antimicrob Agents Chemother 2006; 50;1546-1549.
[11]. Ahamed A,Lumb V,Das MK,Deb V,et al. Prevalence of mutations associated with higher levels of sulphadoxine-pyrimethamine resistance in p.falciparum isolates from Car Nicobar Islands and Assam, India. Antimicrob Agents Chemother2006; 50; 3934-3938.
[12]. Alam TM, Bora, Bharti PK, Saifi MA.,Das K,Deb V,KumarA, Singh N, Dash AP,Das B, Wajihullah,Sharma Y.D.Similar trends of pyrimethamine resistance-associated mutation in p.vivax and P.falciparum. Antimicrob Agents Chemother 2007;51:857-863.
[13]. White NJ, Van Vugt M, Ezzet F.Clinical pharmacokinetics and pharmacodynamics of artemether-lumefantrine. Clin Pharmacokinet 1999; 37:105-125.
[14]. Fried M, Muehlenbachs A, Duffy PE. Diagnosing malaria in pregnancy:an update.. Expert Rev Anti Infect Ther.2012;10(10):1177-1187
[15]. Pharmacological Reviews, March2005 vol 57,no 1 , 117-145

. Roy Sasmit. ""Effectiveness of Artesunate and Mefloquine combination therapy in the treatment of uncomplicated Plasmodium falciparum Malaria in Eastern India: A cross sectional study".". " IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 11, 2018, pp 59-66..