



Effect of Standardized Anti-malarial Herbal Tea (Malatreat) on *Plasmodium berghei* Infection in Mice

**Salome Ida-Awaji Eyaete¹, Hope Delesi Kagbo^{2*}
and Florence Onyemachi Nduka¹**

¹Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Nigeria.

²Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SIAE and HDK designed the study and wrote the protocol. Author SIAE wrote the first draft of the manuscript. Authors HDK and FON performed the statistical analyses and managed the analyses of the study. Author FON managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the curative effect of standardized anti-malarial herbal tea, Trade Name: Malatreat composed of 5 different herbs as follows: *Sida acuta* 10%, *Tridax procumbens* 15%, *Alstonia boonei* 35%, *Phyllanthus amarus* 25% and *Citrus Sinensis* 15% on malaria parasitemia in mice.

Study Design: Animals were separated into five groups of four animals each and housed in plastic cages. The animals were inoculated with *Plasmodium berghei* and treated with the standardized anti-malarial herbal tea, Trade name: Maltreat.

Place and Duration of Study: Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, between 8th September - 11th October 2017.

Methodology: A total of 20 mice were weighed and divided into five groups, 1, 2, 3, 4, 5 of 4 mice each and used for this curative investigation. A pure strain of *Plasmodium berghei* was obtained

*Corresponding author: E-mail: brighthope@rocketmail.com;

from Malaria and Phytomedicine Research Centre, University of Port Harcourt. Five mice were inoculated with an isotonic solution of this pure strain *Plasmodium berghei* infected blood of 68% parasitaemia level and served as stock animals with the infection. All the animals in the various groups were inoculated with 0.5 ml of an isotonic solution composed of 1ml of blood from the stock (an infected animal with parasitaemia level of 75%), and 5ml of buffer and treatment commenced orally on the fourth day after inoculation.

Results: Parasitaemia levels were recorded on the third day after inoculation as follows; 12.06%, 7.95%, 13.62%, 20.1% and 16.05% for groups 1, 2, 3, 4 and 5 respectively. Parasitemia levels had a peak of 15.55%, 20.7%, 25.2% and 21.3% respectively in groups 1, 2, 3, and 4 on 7th-day post-treatment. An observed ($P = .05$) reduction in the parasitaemia levels of the animals in groups 1, 2, 3 and 4 occurred 14th-day post-treatment as follows; 10.16%, 9.36%, 11.82%, 7.9% respectively while group 5 had an increase to 68.75%. The parasite clearance levels in Groups 1 and two at day 14 post-treatment when compared to that of the standard drugs were highly significant ($P = .05$).

Conclusion: These results show that the herbal tea Malatreat (in two different doses) has the potential to reduce parasitaemia level in the blood stream of mice and has a parasite clearance level similar to the reference drugs Chloroquine and Coartem used in this study.

Keywords: Malaria; Malatreat; parasitaemia; *Plasmodium berghei*.

1. INTRODUCTION

Malaria is a major cause of morbidity and mortality world-wide; annually it affects more than 240 million people and is responsible for 600-800 thousand death [1]. Efforts at its control and possible elimination are being made globally, and these include phytomedicine research among others. Many naturopaths believe in the efficacy of herbs to reduce the malaria scourge in Nigeria, and many herbal remedies for malaria abound in the country with varied unverified therapeutic claims. These herbal remedies are highly patronized mainly because they are cheaper than standardly recommended antimalarials especially, ACTs. Maltreat is one of such herbal remedies, a standardized herbal tea registered for human use by the regulatory body in Nigeria with NAFDAC Reg. No: A7-0452L. However, there is a disclaimer by the regulatory body that the claims on this tea as an antimalarial have not been verified. In order to bridge this gap, this study, therefore, set out to investigate the curative effect of this standardized anti-malaria herbal tea, Trade Name: Malatreat composed of 5 different herbs as follows: *Sida acuta* 10%, *Tridax procumbens* 15%, *Alstonia boonei* 35%, *Phyllanthus amarus* 25% and *Citrus Sinensis* 15% on malaria parasitemia in mice.

2. METHODS

The method of Peter [2] was used for the inoculation of parasites into the experimental animals. The parasite used was a chloroquine-sensitive strain of *P. berghei* NK 65 [3],

maintained in mice. Although *Plasmodium berghei* is generally used in rodent model for malaria [4], mice model was used in this study because of the high susceptibility of mice to *P. berghei* infection compared to laboratory rats and hamster, which are less susceptible [5]. *Plasmodium berghei* was obtained from Malaria Research Laboratory, Centre for Malaria Research and Phytomedicine, University of Port-Harcourt. The parasites were kept alive by continuous intraperitoneal injection in mice. Before the beginning of the study, one of the infested mice was kept and observed to show disease symptoms similar to human infection from which other animals were inoculated. The animals weighed between 20 g - 30 g before inoculation, and this weight was used to determine the dose to be administered. All the animals in the various groups were inoculated with 0.5 ml (approximately 1×10^6 pRBCs) of an isotonic solution composed of 1ml of blood from the stock (an infected animal with parasitaemia level of 75%), and 5 ml of buffer and treatment commenced orally on the fourth day after inoculation.

Groups 1 and two were treated with Malatreat herbal tea at concentrations of 1 mg/ml and 0.6 mg/ml respectively. Groups 3 and four were treated with a standard concentration of 5 mg/kg for Chloroquine and ACT, Coartem® respectively that acted as reference drugs while Group 5 had water only. Chloroquine concentration may be determined by the level of inoculum passed into the mice. 20 mg, 10 mg, 5 mg or 2.5 mg/kg have been used in different research [6] and the minimum effective dose is 10 mg/kg [7].

Table 1. Curative investigation of Malatreat

Groups	No. of animals	Treatment
1	4	Maltreat (1 mg/ml)
2	4	Maltreat (0.6 mg/ml)
3	4	Chloroquine (5 mg/kg)
4	4	ACT(Coartem®) Artemether/Lumefantrine (20 mg/120 mg/kg)
5	4	Water

However, in principle, it seems that increasing drug concentrations eventually lead to better therapeutic resolution [6].

Their parasitaemia levels were recorded on days 3, 7 and 14 post treatments for all the groups. Data were analyzed using one-way ANOVA to test differences between groups, and the Least Significant Difference (LSD) was confirmed using Statistical Package for Social Sciences (SPSS) IBM, Version 21. Table one describes the curative investigation.

3. RESULTS

3.1 The Curative Activity of Malatreat Herbal Remedy

Maltreat herbal remedy produced significant ($P = .05$) reduction in parasitaemia levels after three days of treatment with a similar reduction in the reference drugs; Chloroquine and Coartem (positive control). However, there was an increase in parasitaemia on the 7th-day post-treatment across the groups ($P = .05$). It also produced significant ($P = .05$) reduction in

parasitaemia levels 14 days post-treatment with a similar reduction with the referenced drugs; Chloroquine and Coartem® of groups 4 and five as shown in Table 2.

For all the groups, there was an increase in the parasitaemia levels seven days post treatment which was the peak of the infection. See Plate 1.

4. DISCUSSION

These results clearly show that the herbal tea Malatreat (in two different doses 0.1ml and 0.06ml) has the potential to reduce parasitaemia level in the blood stream of mice and has a parasite clearance level similar to the reference drugs Chloroquine and Coartem® used in this study (Plate 2). This was statistically significant using Analysis of variance (ANOVA) and confirmed with SPSS to determine the Least Significant Difference (LSD) of the data generated. Efforts are needed to harness the curative properties of this affordable herbal tea in follow up studies and possible clinical trials in the country to enhance malaria control efforts and possible elimination.

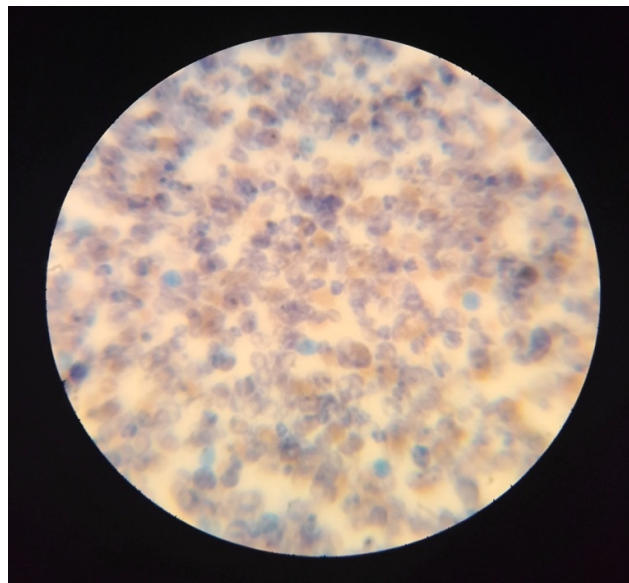


Plate 1. Showing the peak of parasitaemia in the red blood cells of all the groups

Comparing Malatreat groups 1 and 2 with the reference drugs chloroquine and Coartem® and control, 7th and 14th-day post-treatment showed

the LSD significant at P= .05. This can be described in Figs. 1, 2, 3 and 4.

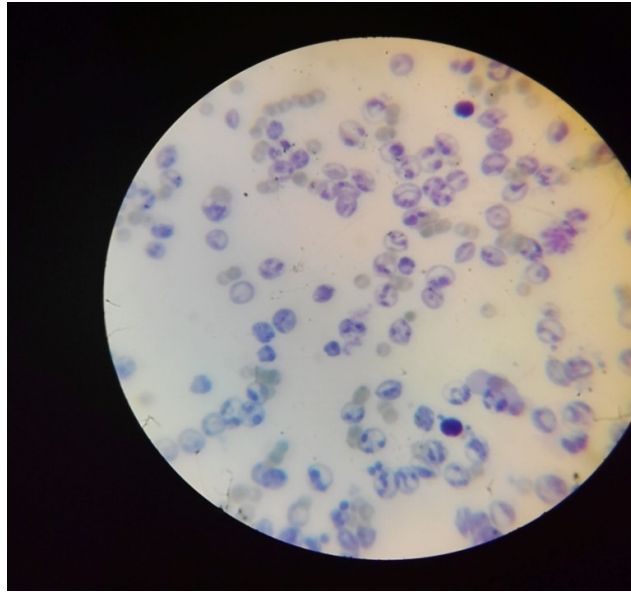


Plate 2. Parasite clearance observed in the Malatreat and reference drug groups

Table 2. The curative activity of Malatreat herbal remedy on *Plasmodium berghei*-infested mice

Groups	In %	Three days %	7 days %	14 days %
Control	22.74	25.93	30.56	67.48
Maltreat 1 (0.1 ml)	12.06	14.88	20.71	10.16
Maltreat 2 (0.06 ml)	7.95	7.63	15.59	9.31
Standard 1 Chloroquine (5 mg/kg)	23.26	15.74	25.3	10.75
Standard 2 Coartem® Artemether/Lumefantrine (20 mg/120 mg)	28.9	18.38	18.79	7.35

Ini= Initial parasitaemia level; *Three days* = Parasitaemia level 3 days post-treatment; *Seven days*= Parasitaemia level 7 days post-treatment; *14 days*= Parasitaemia levels 14 days post-treatment

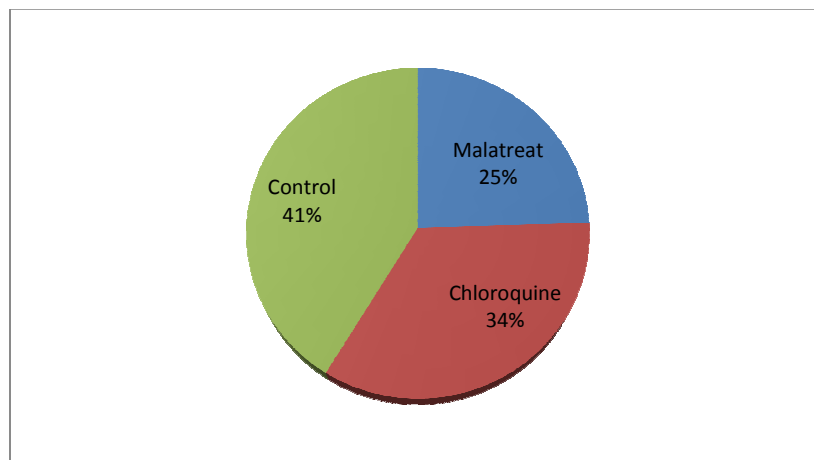


Fig. 1. Parasitaemia level in Maltreat, chloroquine and control groups, 7th-day post-treatment

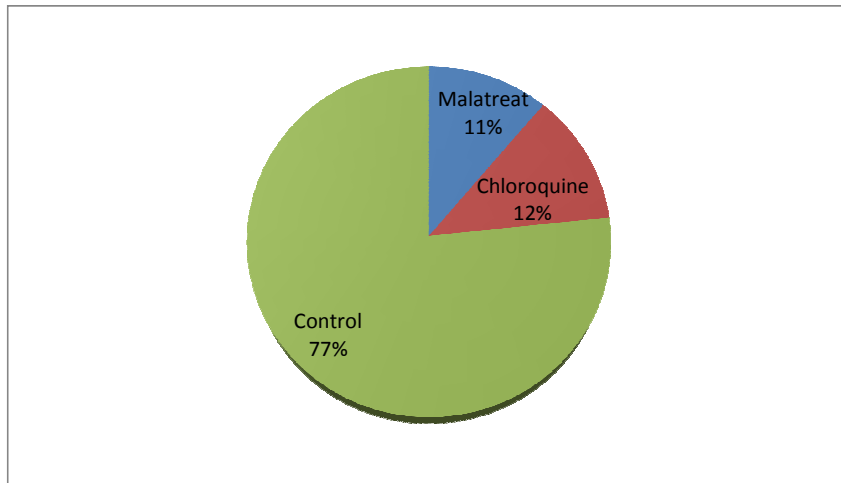


Fig. 2. Reduced parasitaemia observed in Malatreat and Chloroquine groups and parasitaemia in the negative control group

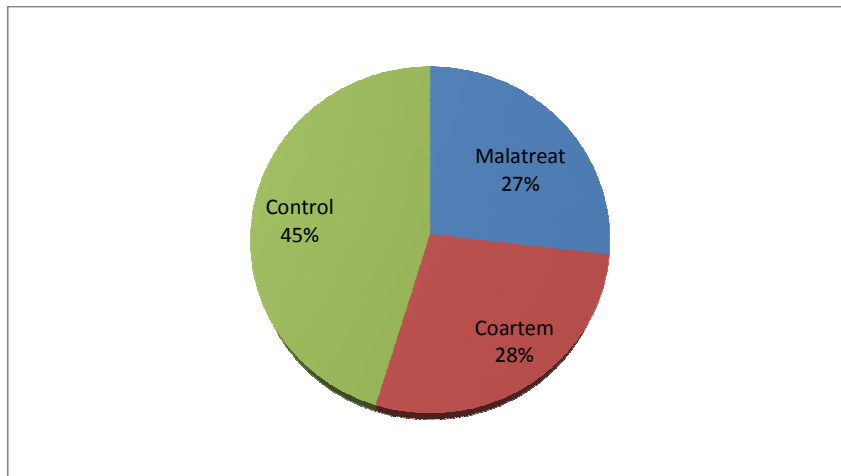


Fig. 3. Parasitaemia levels in Maltreat, Coartem and negative control groups

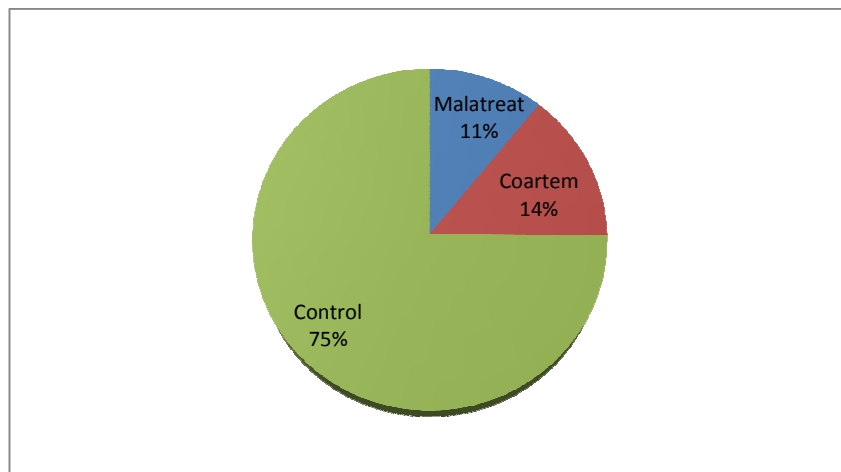


Fig. 4. Parasitaemia levels in Maltreat, Coartem® and negative control groups

5. CONCLUSION

Maltreat herbal tea exerted a curative activity against *Plasmodium berghei* infested mice. This was statistically significant at $P = .05$. To ensure elimination of malaria, the grass-root level must seriously be considered. Due to inaccessibility of health facilities, a lot of people die in rural places because they do not know the potency of the herbs at their backyards. If these herbs in our environment are carefully combined, useful medicines at low cost would be produced. After all, Artemisinin-based combination therapy (ACTs) are products of herbs found in some parts of the world. *Sida acuta* 10%, *Tridax procumbens* 15%, *Alstonia boonei* 35%, *Phyllanthus amarus* 25%, *Citrus Sinensis* 15%, were combined to produce Malatreat Tea.

ETHICAL APPROVAL

Ethical approval was sought for this study and was approved by the Ethical Committee of the University of Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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