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Research Article

Anti-plasmodial Activity of Chloroform Leaf Extract of *Eucalyptus camaldulensis* in Mice

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ABSTRACT

Malaria outbreak has been a case of concern for quite a long time now. With the ever increasing resistance to the available standard antimalarial drugs, there is need for the development of new antimalarial drugs, hence, the purpose of this research is to evaluate the antiplasmodial activity of the chloroform leaf extract of *Eucalyptus camaldulensis* at various dosage concentrations in vivo compared to chloroquine treatment in experimental mice infected with chloroquine sensitive *Plasmodium berghei* NK65 strain. Groups A, B and C mice were infected and treated with 100, 200 and 300 mg extract/kg body weight of mouse respectively while group D was infected and treated with 25mg chloroquine /kg body weight, group E was infected but not treated and group E was unfected, untreated. The phytochemical constituents of the plant extract were evaluated giving 8 phytochemicals including flavonoids. In addition, the extract indicated a dose dependent decrease in the level of parasitaemia. The analysis showed that the extract has no effect on the packed cell volume of the experimental mice. Also, some of the groups lived 10 days beyond the experimental period. Furthermore, the body weights of the groups treated with 100mg/kg and 300mg/kg extract reduced compared to the infected untreated group whereas, the groups treated with 200mg/kg extract, 25mg/kg chloroquine and uninfected untreated increased in body weight compared to the negative control (infected untreated). Therefore, the chloroform leaf extract of *Eucalyptus camaldulensis* could serve as a possible source of antimalarial compounds.

Keywords: *Plasmodium berghei*; malaria; *Eucalyptus camaldulensis*; antiplasmodial; chloroform extract

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INTRODUCTION

Malaria is one of the major health threats to populations in tropical and subtropical poor countries in the World such as Nigeria (Okeke, 2012). At present, around 3.2 billion people are at risk of malaria each year globally (WHO, 2005), with 2-3 million deaths occurring each year (Snow *et al*., 2015). Malaria can be, in certain epidemiological circumstances, a devastating disease with high morbidity and mortality demanding a rapid and comprehensive effort (Bloland, 2011). It is a common and devastating disease caused by protozoan parasites of the genus Plasmodium. These parasites are spread through the bites of Anopheles mosquitoes. There are four known species of *Plasmodium* that infect humans, these are: *P. falciparum, P. vivax,P. malariae* and *P. ovale*. Out of these four species, P. *falciparum* is known to be responsible for majority of the severity and loss of lives associated with malaria (Bhatt *et al*., 2015).

In many developing nations of the world, large numbers of people still rely heavily on traditional methods and medicinal plants to meet their daily primary healthcare needs and overcome the problems of resistance and side effects of the currently available antimicrobial agents (Qais *et al*., 2011). Right from its beginning, the documentation of traditional knowledge, especially on the medicinal uses of plants, has provided many important drugs of modern day (Cox and Balick, 1996; Flaster, 1996). In Africa, the use of indigenous plants still plays an important role in malaria treatment and these plants might be interesting sources for the detection of novel antiplasmodial compounds[6].

The rapid spread of resistant parasite, makes it necessary to search for more effective antimalarial compounds and among local medicinal plants for possible anti-malarial properties. Therefore, the importance of these medicinal plants cannot be overemphasized owing to the fact that their active components are very important in the treatment of these tropical ailments such as malaria, diarrhea, etc.

Eucalyptus camaldulensis (Myrtaceae) also known as river red gum, an Australian native, represented by around 700 species is a genus of 20m to 50m tall, evergreen and

magnificent trees cultivated in the world over for its oil, gum, pulp, timber, medicine and aesthetic value. Among the various wood and non-wood products, essential oil found in its foliage is the most important one and finds extensive use in food, perfumery and pharmaceutical industry. It is a plantation species in many parts of the world but is native to Australia, where it has the most widespread natural distribution of Eucalyptus in Australia (Colloff, 2014). It has smooth bark to the smaller branches ranging in colour from white and grey to red-brown, frequently with loose basal slabs in the lower trunk, and which it sheds in large plates or flakes or short ribbons (Mullins, 2012). In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal (Batish *et al*., 2008). *Eucalyptus* oil is readily steam distilled from the leaves and can be used for cleaning, deodorising and in very small quantities in food supplements; especially sweets, cough drops and decongestants. Eucalyptus oil has insect repellent properties and is an active ingredient in some commercial mosquito repellents (Doran and Brophy, 1990). *Eucalyptus camaldulensis* consists of two variations and one subspecies: *E. camaldulensis var. camaldulensis*, and *E. camldulensis var. obtusa*. The subspecies *Eucalyptus simulata*, found in North Queensland, has been recognised as a hybrid of *var. obtusa* and *Eucalyptus tereticornis* (Gippel *et al.*, 2014).

However, in this work, the components in the chloroform extract of *Eucalyptus camaldulensis* leaves were tested to check for their anti-malarial properties in experimental mice.

MATERIALS AND METHODS

Plant Collection, Preparation and Extraction

Fresh leaves of *Eucalyptus camaldulensis* were collected from the University of Jos senior staff quarter on the 10th of May 2018, subsequently air dried at room temperature and pounded into its powdered form using mortar and pestle, and stored in an air-tight container prior to extraction.

100g of the plant leaf powder was placed into a 500ml conical flask and constituted with 300ml absolute chloroform. This constituted plant material was left to stand for 48hours and mixed thoroughly for 1hour for proper extraction. The content was filtered using a Whattmann filter paper placed on a Buchner funnel-flask using a vacuum pump. The residue was subjected to several parts of rinsing and filtration using fresh chloroform to attain some level of exhaustive extraction. The filtrate was air-dried using an evaporating dish. The dried extract was harvested and stored in air-tight container for subsequent phytochemical analysis and anti-plasmodial assay in experimental mice.

Phytochemical Analysis for the Plant Extracts

The chloroform extract of *Eucalyptus camaldulensis* was subjected to phytochemical screening to check for the presence or absence of plant secondary metabolites such as: Saponins, tannins, alkaloids, flavonoids, steroids and terpenes, cardiac glycosides, balsam, carbohydrates, phenols and resins according to the method of Trease and Evans, (1983) with slight modification.

Preparation of Experimental Mice (Grooming and Infection)

Ethical clearance was obtained with approval number UJ/FPS/F17-00379 and white albino mice species which has susceptibility to the *Plasmodium berghei* NK65 were obtained.

The experimental mice were obtained from the animal house in the University of Jos on the 5th of July, 2018. They were 21 days old as at the time of purchased and they were well fed and looked after for about two more weeks before infection so as to add more weight. The experimental mice were separated into six groups with three mice in each group. The infectious *Plasmodium berghei* NK65 strain parasite was obtained from Malaria Research Institute Ibadan, inoculated into experimental mice and transported to the animal house at the University of Jos, where this research took place. About 3mls of blood from the infected mice was collected and mixed with 0.9ml of normal saline. The uninfected mice were inoculated intraperitoneally with 0.5ml parasite suspension.

Experimental Design

1. GROUP A: 3 parasitized mice administered 100mg/kg of extract per body weight per day.
2. GROUP B: 3 parasitized mice administered 200mg/kg of extract per body weight per day.
3. GROUP C: 3 parasitized mice administered 300mg/kg of extract per body weight per day.
4. GROUP D: 3 parasitized mice administered 25mg/kg of chloroquine per body weight per day.
5. GROUP E: 3 parasitized mice with no treatment.
6. GROUP F: 3 uninfected and untreated mice.

Mode of administration; oral administration.

Blood Sample Collection for Parasitemia Screening

After 48 hours of infection, blood samples were collected from the infected mice to check for the presence of the parasites. Pricking the tip of the tails of the mice caused blood to flow which was collected on slides and smeared to make a thin blood film and dried under the shade for about 45 minutes and was fixed with methanol. After the methanol dried completely, the blood was stained with giemsa for 10 minutes and rinsed with water then taken for microscopic examination after drying.

Immersion oil was added to the stained slides and viewed under the X100 objective lens. The *Plasmodium berghei* were seen in the infected blood samples as spherical, curved or clustered images in the stained samples. Parasitemia was counted based on giemsa positive bodies which represents the parasitized red blood cells. The parasitemia count was done and recorded 48 hours after infection and daily throughout the period of the experiment.

RESULTS

Phytochemical Screening

The percentage yield of the chloroform leaf extract of *Eucalyptus camaldulensis* was calculated to be 3.30% and the result was given in Table 1. Table 2 shows the results obtained from the phytochemical screening of the chloroform extract of powdered leaf of *Eucalyptus camaldulensis* showing

alkaloids, flavonoids, tannins, terpenes and steroids, balsam, phenol, resins, and cardiac glycosides to be present.

Mortality Rate Post Infection

The death rate of the experimental albino mice were monitored daily after the inoculation of *Plasmodium berghei*. Groups A, B, C, and E lived upto day 21, 14, 17, and 14 respectively meanwhile groups D and F lived beyond the experimental period as shown in Table 3.

Differences in Body Weight

Effects of chloroform leaf extract of *Eucalyptus camaldulensis* on the body weight of albino mice infected with *Plasmodium berghei* were shown in Figure 1. The weight of the groups treated with 100mg/kg extract, 300mg/kg extract, 25mg/kg chloroquine and the group infected but untreated reduced by 12.9g, 6.3g, 2.1g and 3.3g respectively. Whereas, the weight of the groups treated with 200mg/kg extract and uninfected and untreated increased by 0.4g and 5.2g respectively.

Parasitaemia Count

The average daily parasitaemia level of the *Plasmodium berghei* in infected mice treated with chloroform leaf extract of *Eucalyptus camaldulensis* are shown in Figure 2. The average daily parasitaemia of infected mice treated with 300mg/kg of *Eucalyptus camaldulensis* chloroform leaf extract and 25mg/kg of chloroquine significantly ($P<0.05$) reduced when compared with negative control group. However there was no significant ($P>0.05$) difference in the level of parasitaemia in infected mice treated with 100 mg/kg and 200mg/kg chloroform leaf extract of *Eucalyptus camaldulensis* as compared with the negative control group.

Table 1:
Percentage Yield of Chloroform Extract

Eucalyptus camaldulensis	Weight(g)
Leaf extract	100.00
Chloroform extract	3.30
Extract yield(%w/w)	3.30

Table 2:
Phytochemical profile of chloroform extract of *Eucalyptus Camaldulensis* Leaf

PHYTOCHEMICAL	RESULT
Alkaloid	+
Flavonoids	+
Tanins	+
Saponins	-
Terpenes and steroids	+
Cardiac glycosides	+
Balsam	+
Carbohydrate	-
Phenol	+
Resins	+

KEY: + = Present - = Absent

TABLE 3:
Survival rate post infection

GROUPS	SURVIVAL RATE
100mg/kg extract	21
200mg/kg extract	14
300mg/kg extract	17
25mg/kg extract	Lived beyond experimental period
Infected, untreated	14
Uninfected untreated	Lived beyond experimental period

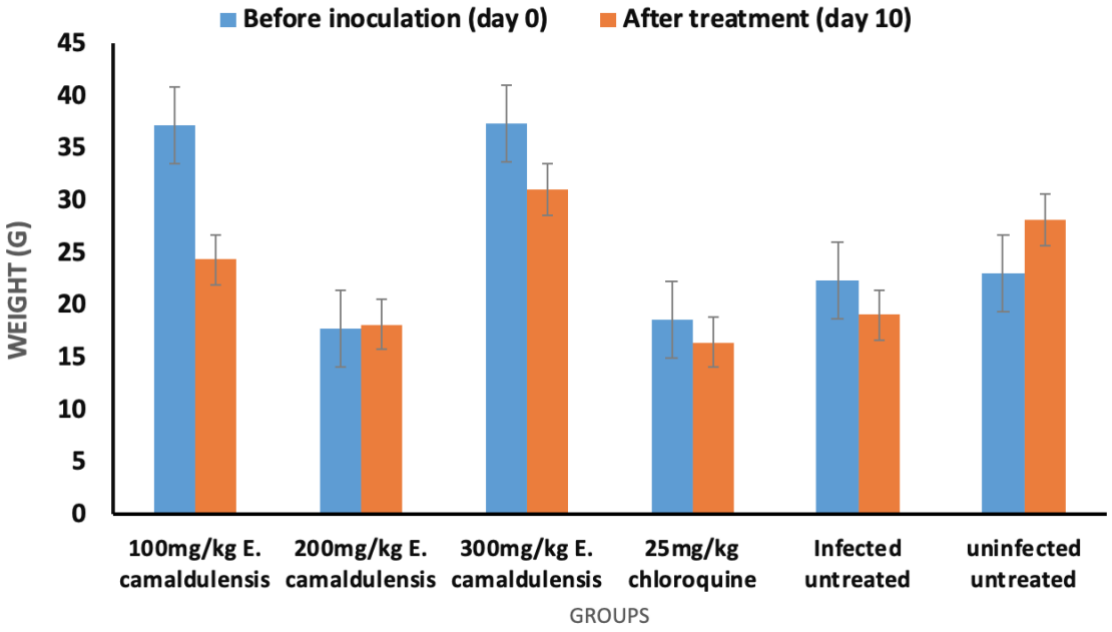

Figure 1:
Average mean weight of mice before inoculation of parasite and after administration

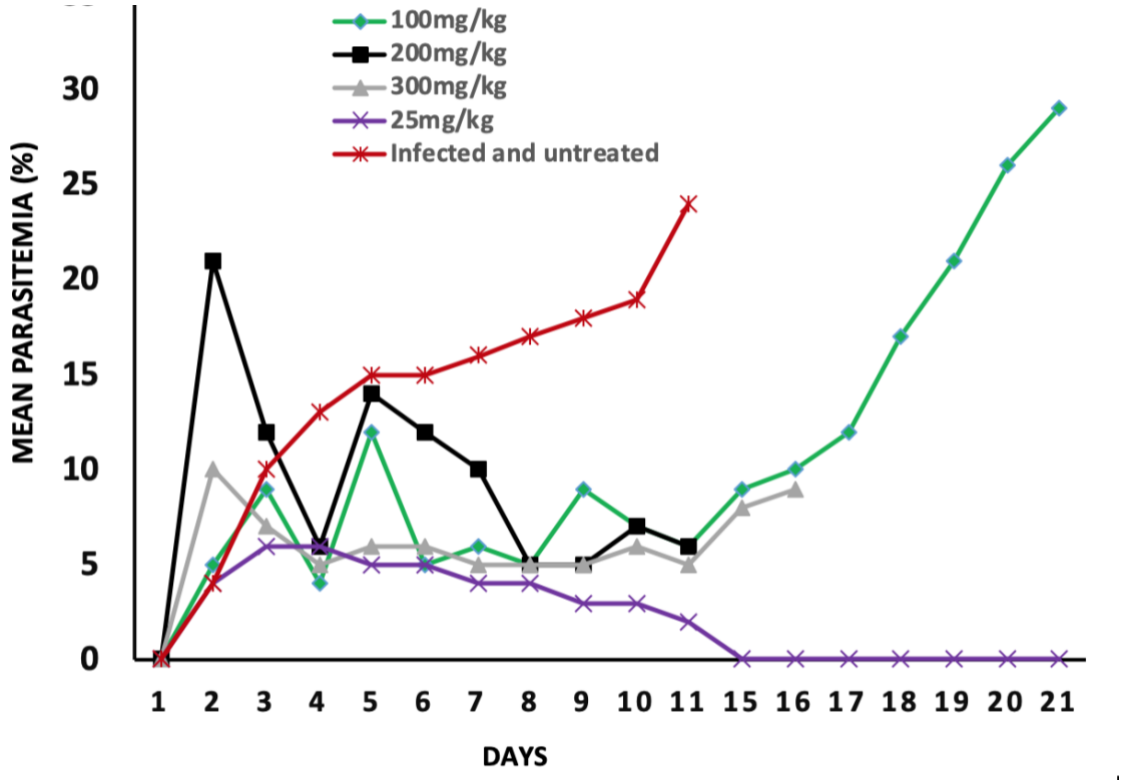

Figure 2:
Average mean parasitemia count from day one of infection

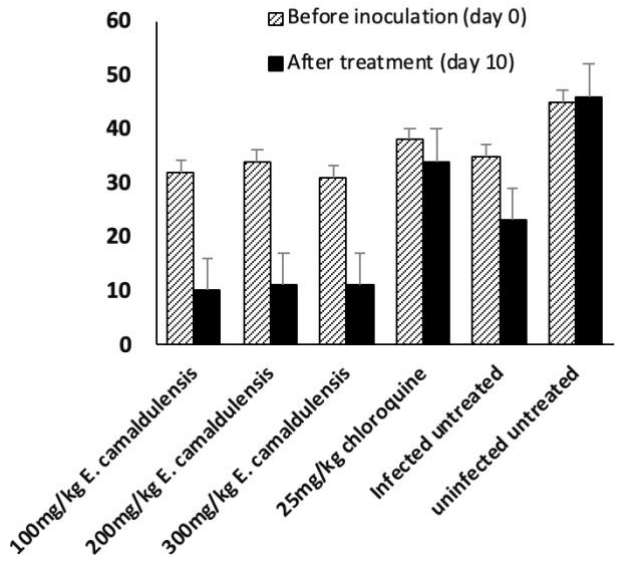

Figure 3:
The packed cell volume of mice before inoculation of *Plasmodium berghei* and after treatment

Packed Cell Volume

Effects of chloroform leaf extract of *Eucalyptus camaldulensis* on packed cell volume of *Plasmodium berghei* infected mice were shown in Figure 3.

The percentage packed cell volume of the groups treated with 100mg/kg extract, 200mg/kg extract, 300mg/kg extract, 25mg/kg chloroquine, and infected but untreated reduced by 22%, 23%, 20%, 4%, and 12% respectively whereas, the group uninfected and untreated increased by 1%.

DISCUSSION

The pulverized leaf of *Eucalyptus camaldulensis* was extracted using absolute chloroform and the extract subjected to *in vivo* anti-plasmodial studies compared to chloroquine, a standard anti-malaria drug showed dose dependent anti-plasmodial activities against the *Plasmodium berghei* species in experimental mice. The various dosage concentration of 100mg/kg, 200mg/kg and 300mg/kg per body weight all showed curative activity of the chloroform leaf extract of *Eucalyptus camaldulennsis* and chloroquine to different extents. However, the 25mg/kg of chloroquine and the 300mg/kg dosage concentration of the chloroform leaf extract of *Eucalyptus camaldulensis* showed more curative activities compared to the lower dosage concentration of 100mg/kg and 200mg/kg per body weight. This research study presented that the 200mg/kg of the chloroform extract of *Eucalyptus camaldulensis* significantly prevented weight loss associated with increase in parasitemia level when compared with the negative control group is an indication of ameliorating potentials of the plant extract on the anaemia induced by the malarial infection.

The phytochemical screening of the chloroform leaf extract of *Eucalyptus camaldulensis* showed that the leaf contains useful

phytochemicals which contributed to its anti-plasmodial activities in experimental mice. The presence of alkaloids, flavonoids, tannins, terpenes and steroids, balsam, phenol, resins, and cardiac glycosides in the chloroform extract attributes this anti-plasmodial activities of the chloroform leaf extract of *Eucalyptus camaldulensis*. Flavonoids have been reported to have exhibited significant in vitro antimalarial activity against P. falciparum (Chanphen *et al.,* 1998). This could justify the antimalarial activities exhibited by the plant extract since flavonoids was found to be present in the results of the phytochemical screening.

At the varying concentrations of the chloroform extract's dosage, the leaf of *Eucalyptus camaldulensis* showed varying degrees of treatment of the malaria parasite. Therefore, this shows that *Eucalyptus camaldulensis* has both curative and suppressive activities since at lower concentrations, the parasite load was seen to reduce from the initial load before administration and at higher concentrations, the parasite was seen to reduce to the lowest level indicating that if treatment period is extended or higher dosage concentration is administered, the parasite will be cleared completely, thus, the curative activities of the *Eucalyptus camaldulensis* leaf extract.

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