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Activity of Chloroform Extract of *Eucalyptus camaldulensis* Root against *Plasmodium Berghei* in vivo

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Abstract

The work was designed to investigate the anti-malarial activity of chloroform root extract of *Eucalyptus camaldulensis* on *Plasmodium berghei* NK65 strain infection *in vivo*. Eighteen mice were intraperitoneal infected with chloroquine sensitive *P. berghei* strain and shared into 6 equal groups. Groups A, B, and C were treated, after infection, with 100, 200 and 300 mg extract/kg body weight of mouse respectively while group D was treated with 25 mg chloroquine/kg body weight. Group E mice were infected and administered only normal saline (negative control), and group F was neither infected nor treated. Phytochemical constituents of the plant extract were evaluated. The three concentrations of the extract resulted in reduced parasitemia, although the 200 mg/kg administered to group B had more effect than the 300mg/kg and 100 mg/kg administered to group C and A respectively. The highest activity was observed in the chloroquine group (positive control group). Also, at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg, the extract produced increase in body weight and life span as compared to mice in the negative control group. At doses of 100mg/kg and 300mg/kg, the extract produced increase in PCV of the infected mice as compared to mice in the negative control group. Phytochemical screening showed that the leaf extract contains alkaloids, Balsam, Resin, cardiac glycosides and terpenes and steroids. The chloroform root extract of *Eucalyptus camaldulensis* presented a transient effect on *Plasmodium infection* in mice and so justifies the use of the plant as part of native desertion against malaria.

Keywords: *Plasmodium berghei;* malaria*; Eucalyptus camaldulensis.*

1. Introduction

Malaria is a vector–borne infectious disease caused by eukaryotic protists of the genus Plasmodium. There are four types of *Plasmodium* species that caused malaria namely, *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium ovale*, and *Plasmodium Malariae*. It is a disease transmitted by infected female Anopheles mosquito (Toure et al., 2004). A *Plasmodium* Spp depends on two hosts to complete its life cycle, a female anopheles mosquito and a human. The disease is widely spread in tropical and sub-tropical regions, including most of sub-Saharan Africa, Asia and America. Malaria is prevalent in these regions because of the significant amount of rainfall, warm temperatures, and high humidity, along with stagnant waters that provide the mosquitoes with the environment needed for continuous breeding (Prothero; Mansall, 1999).

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It is estimated that about 81 % of all malaria deaths in the world occur in African region. Majority of infections in Africa is caused by *Plasmodium falciparum*, the most lethal of the four human malaria parasites. About 655,000 people die of malaria, 91 % in African region, and 86 % of this are children under the age of five. Malaria is a risk of 97 % of Nigeria's population. There are estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria (WHO, 2016). Nigeria's ministry of health reported in April 2004 that malaria is responsible for one out of ten deaths in pregnant women and cost the federal government over one billion naira annually. The report added that Nigeria is often associated with poverty which is the major hindrance to economic development, despite the effort made to reduce the transmission; there has been little change in areas at risk of this disease since 1992. The precise statistics are unknown because many cases occur in rural areas where people do not have access to the hospital or the means to afford health care (Hay et al., 2008). Malaria has infected humans for over 50,000 years and may have been a human pathogen for the entire history of humans (Joy et al., 2003).

Anti-malarial drugs have been used in various ways to prevent or treat malaria infection in the resident populations of malaria-endemic' areas for nearly 100 years (Greenwood, 2004). However the continual resistance of the parasite especially *Plasmodium falciparum* to currently used anti-malarial drugs make it imperative to search for newer and more effective therapies. Quinin was used in the seventeenth century as a prophylactic against malaria followed by the development of quinacrine, chloroquin, and primaquin were used in the twentieth century to reduce the reliance on quinine (WHO, 2006). The World health organization initiated the based combination therapy, (artemisinin), obtained from *Artemisia annua* (a plant in china), and quinine to treat malarial infection (WHO, 2007).

Eucalyptus camaldulensis is found in many parts of the world. It has been use in Australia as a source of honey (Lupo, Eisikiowitch, 1989). The leaves are used to treat trypanosomiasis (Kabiru et al., 2012). Phytochemical screening revealed the presence of terpenes, steroids, tannins, alkaloids, and fatty acid. It possess anti-inflamatory, analgesic, antimicrobial, antiparasitic, antioxidant, antidiabetic, anti-insecticide, repellent, dermatological and anti cancer effect (Arl-Snafi, 2015). Therefore, the rat was studied against malaria parasite in this study.

2. Materials and methods

Plant Collection, Preparation and Extraction

Roots of *Eucalyptus camaldulensis* were collected from university of Jos senior staff quaters, and were authenticated by a Botanist in the College of Forestry Jos.The roots were washed with clean-water, air dried to constant weight and milled using a mechanical grinder. Extraction of plant material was performed by soxhlet apparatus (Sigma-Aldrich, USA) using chloroform. The extract was stored in a Refrigerator until required. About 100 g of the extract was put into a conical flask and soaked in 500 ml of 99 % chloroform. This was then left for 24 hours. The mixture was filtered using a white cloth and Whatmann filter paper. The filtrate was then kept in the laboratory oven at 40^{o} C to dry. The dried extract was used for phytochemical analysis and anti-plasmodial assay in experimental albino mice.

Phytochemical Analysis of the Plant Extracts

The chloroform extract was subjected to phytochemical screening to detect the presence or absence of plant secondary metabolites: alkaloids, Balsam, Resins, terpenes and steroids, and cardiac glycosides according to the method of Trease and Evans (1987).

Parasite Inoculums

Plasmodium berghei infected erythrocytes were obtained from a donor-infected mouse at malaria research institute, Ibadan and maintained at animal farm University of Jos, Plateau state, Nigeria. The inoculum was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and then diluting with normal saline.

Experimental Animal and Curative Test

The eighteen (18) albino mice weighing between 18.6-25.6 g used in this study were obtained from the animal house of University of Jos, Plateau State, Nigeria. They were kept in plastic cages

with saw dust bed and given standard laboratory chore and water. They were then allowed to acclimatize for two weeks to their new environment before the initiation of the experiments. In order to evaluate the antimalarial potential of the crude extract, methods described (Akuodor, Idris, 2011; Ryley, Peters, 1995) in literature were adopted. Each mouse in the treatment group was inoculated intraperitoneal with infected blood suspension (0.3 ml) containing *Plasmodium berghei* parasitised red blood cells on day zero. Groups A, B, and C were dosed once daily for ten days with 100, 200 and 300 mg/kg/day of the chloroform root extract respectively. Chloroquine diphosphate (25 mg/kg body weight/day) was administered to group D mice and 0.2 ml normal saline to group E mice (negative control group). All treatments were orally done for ten days from when parasites were first seen in the infected animal blood.

Parasitemia Count

On each day of treatment, a drop of blood was collected from the tail of each infected mouse for parasitemia screening. The blood collected was placed on a slide and smeared to make a thick film, fixed with methanol and stained with Giemsa stain and air-dried. The film was microscopically viewed by adding a drop of immersion oil and viewed under x100 magnification of the microscope. The parasitemia density was examined by counting the parasitized red blood cell.

Determination of Packed Cell Volume

Capillary tubes were filled with blood to about 1 cm or two-third (2/3) of its length and the vacant end of each sealed with plasticin to protect the blood from spilling. The tubes were placed in haematocrit centrifuge with sealed side towards the periphery and then centrifuge for 5 minutes. The packed cell volume was read directly from haematocrit reader.

3. Results

Extract Yield

The percentage yield of the leaf extract is shown in Table 1. The yield of chloroform root extract of *E. camaldulensis* was 16.5 %. Table 2 shows the result of phytochemical composition of chloroform root extract of *Eucalyptus camaldulensis*. The results revealed the presence of alkaloids, Balsam, Resins, cardiac glycosides, Terpenes and steroids.

Table 1. Percentage yield of chloroform root extract

Root powder	100.00
Chloroform extract	16.50
Extract yield (% w/w)	16.50

Table 2. Phytochemical composition

phytochemicals	Inference
Alkaloid	+
Flavonoids	-
Tannins	-
Saponins	-
Terpenes and steroids	+
Cardiac glycosides	+
Balsam	+
Carbohydrates	-
Phenols	-
Resins	+

Key: (-) absent, (+) present

Parasitaemia Count

The average daily parasitaemia level of the *Plasmodium berghei* in infected mice treated with Chloroform root extract of *Eucalyptus camaldulensis* are shown in Figure 1. The average daily

parasitaemia of infected mice treated, 100, 200, 300 mg/kg of root extract of *Eucalyptus camaldulensis* and chloroquine (25 mg/kg) reduced when compared with control group, although the 200 mg/kg is more effective followed by the 100 then 300 mg/kg. The highest activity was observed for the standard group (chloroquine).

Body Weight

Effect of chloroform roots extract of *Eucalyptus camaldulensis* on body weight of *Plasmodium berghei* infected mice is shown in Figure 2. The body weight of the infected but untreated mice showed a decrease in body weight. Those treated with, 100, 200, chloroquine, and 300 mg/kg respectively showed significant ($P < 0.05$) increase in body weight after 10days post treatment when compare to the infected but untreated group (negative control).

Packed Cell Volume

Effect of chloroform root extract of *Eucalyptus camaldulensis* on PCV of *Plasmodium berghei* infected mice is shown in Figure 3. The PCV of *P. berghei* infected untreated mice gave about % reduced. Those infected and treated with 300, 200, and 100mg/kg of *Eucalyptus camaldulensis* root respectively as well as those treated with 25 mg/kg chloroquine showed various effects on the PCV that are better than that in negative control group. On the other hand, the un-infected untreated mice showed significant increase in PCV after 10 days of treatment.

Effect of *Eucalyptus camaldulensis*' root on survival time

Figure 4 shows the survival time of the mice treated with the various concentrations of the crude extract and those infected, but untreated. The normal control, positive control and the group treated with 100 mg/kg lived beyond the experimental period of twenty one days; the group treated with 300 mg/kg lived for twenty days; the group treated with 200 mg/kg lived for sixteen days, while the negative control lived for fifteen days.

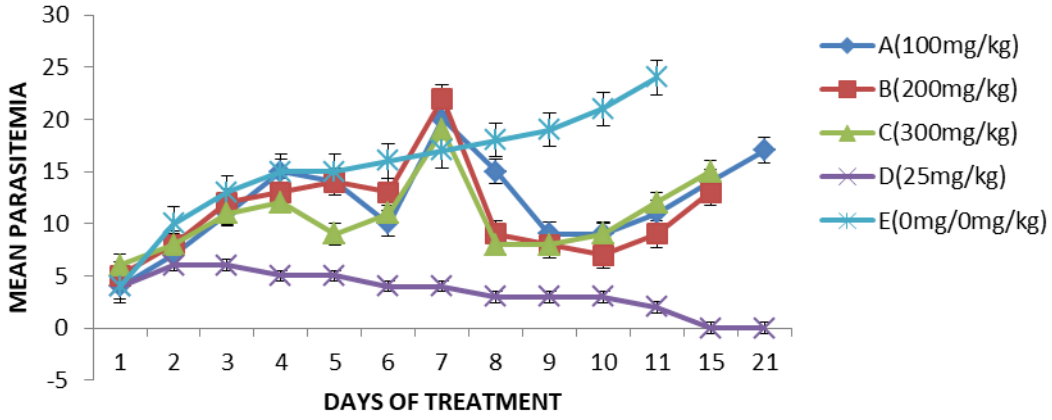

Fig. 1. The parasitaemia levels after treatment with chloroform root extract of *Eucalyptus camaldulensis*

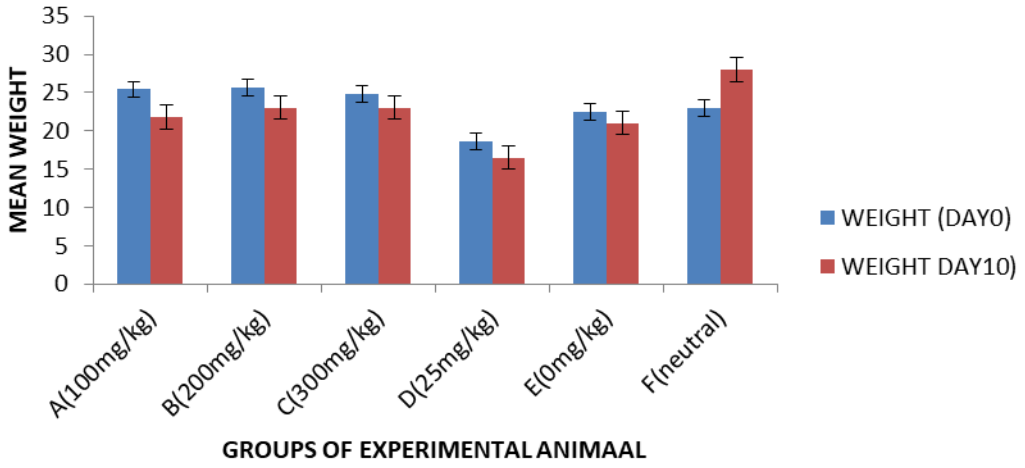

Fig. 2. The mean weight of *Plasmodium berghei* infected mice treated with chloroform root extract of *Eucalyptus camaldulensis*

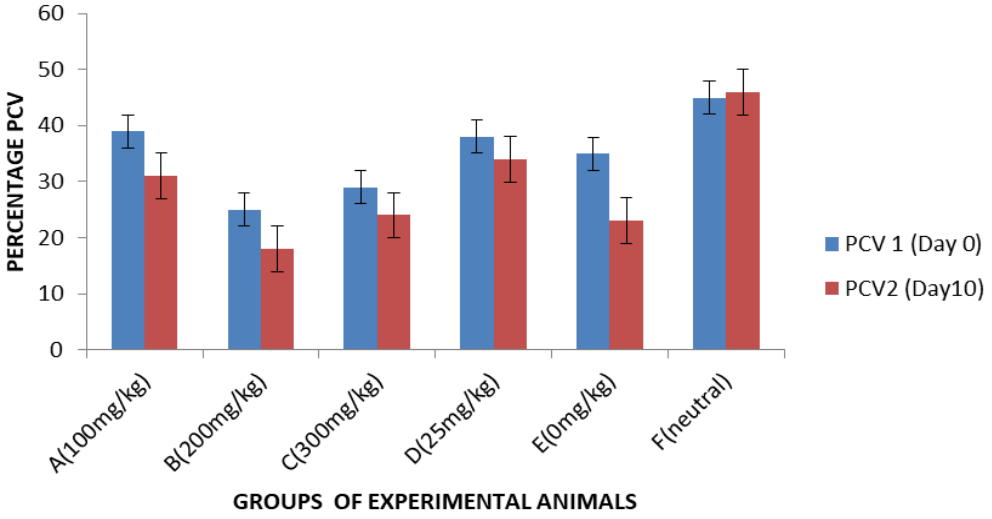

Fig. 3. The mean packed cell volume of *Plasmodium berghei* infected mice treated with chloroform root extract of *Eucalyptus camaldulensis*

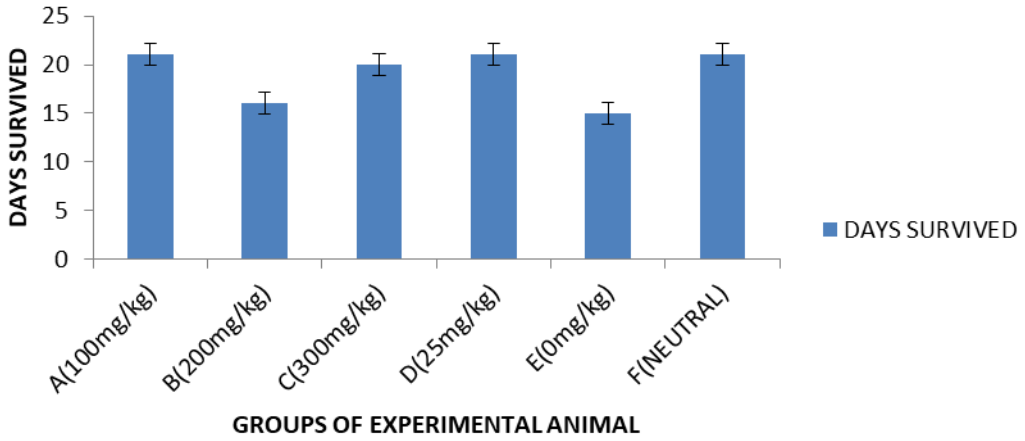

Fig. 4. The survival time after treatment with chloroform root extract of *Eucalyptus camaldulensis*

4. Discussion

Plants used in treatment of diseases are said to contain active phytochemicals some of which are responsible for the plants' characteristic odours, pugencies and colour while others give virtue as food, medicinal or poison (Evans, Evans, 2002). The phytochemical screening of the extract revealed that *Eucalyptus camaldulensis's root* contained some active chemical compounds such as Alkaloids, Flavonoids, Resins, Balsam, cardiac glycoside, Terpenes and Steroids. The presence of secondary metabolites in plants produced some biological activity in man and animals and it is responsible for their used as drugs (Evans, Evans, 2002) and therefore explains its traditional use as health remedy. Secondary metabolites in plants confer protection against bacterial, fungal and pesticidal attacks and thus, are responsible for the exertion of antimicrobial activity against some microorganisms (Marjorie, 1999). Flavonoids have been reported to have exhibited significant *in vitro* antimalarial activity against *P. Falciparum* (Wanauppathamkul, Yuthavong, 1998). This could justify the antimalarial activities exhibited by the plant extract. The 200mg/kg administered to group B has more effect than the 300mg/kg and 100mg/kg administered to group C and A respectively. After treating the curative groups for ten days, parasitemia level was checked on day 11, 15, and 21, and it was observed that the parasitemia level increased in the curative group that means there was a relapse. *Eucalyptus camaldulensis* leaf extract was found in previous studies to be effective against Trypanosoma *brucei* (Kabiru et al., 2012) but from this study it was not effective against *Plasmodium berghei*. The finding in this study did not agree with earlier reports of studies using different extract. Antiplasmodial activity was observed in the ethyl acetate crude extract of C*arica papaya* against *P. falciparum* (Melariri et al., 2011). Unlike *Carica papaya* which was found to be effective against *Plasmodium berghei* in previous study (Longdet, Adoga, 2017), chloroform root extract of *Eucalyptus camaldulensis* has only transient effect on *Plasmodium berghei.* Chloroquine has been used as the standard antimalarial drug because of its established activities on *P. berghei* (Ajaiyeoba et al., 2006). Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice (Langhorne et al., 2002). So an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in infected mice (Bantie et al., 2014). The extract of *E. Camaldulensis* at 100, 200, and 300 mg/kg body weight decreased the weight of the infected mice following ten days of administration after infection. The chloroform root extract of *Eucalyptus camaldulensis* at 200 and 300 mg/kg body weight *prevented* weight loss associated with increase in parasitemia level by 10.2 % and 7.3 % respectively when compared with the positive control. This is an indication of ameliorating potentials of the plant extract on weight loss. Extracts of *Eucalyptus camaldulensis* decrease the level of PCV in infected mice, this is expected as anemia is one of the general features of malaria in infected mice. At 100, 200, and 300mg/kg body weight, the PCV level reduced by 20.5 %, 28 %, and 17.2 % when compared with the negative control group which reduced the PCV level by 34.3 %, we can say that the extract helped in preventing anemia associated with increase in parasitemia.

5. Conclusion

This indicates that *Eucalyptus camalduensis* contains important phyto-constituents that could be implicated in the observed antimalarial effect of the plant. However, the active compound (s) known to give this observed activity need to be identified.

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