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Evaluation of the Antiplasmodial Activity of Diethyl Ether Leaf Extract of Eucalyptus Camaldulensis in Experimental Mice

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Abstract

The main aim of this research was to study the *in vivo* antiplasmodial activity of diethyl ether fraction of *Eucalyptus camaldulensis* leaf on malaria infection in experimental albino mice. The dried leaves were pounded into its powdered form with the aid of mortar and pestle. The plant leaf was extracted with Diethyl ether, yielded 4.03 g extract and the extract was screened for antiplasmodial activity. Mice infected with *Plasmodium berghei* were administered intraperitoneally doses ranging from 100-300 mg/kg body weight/day of the extract for 10 consecutive days. The positive control group was treated with 25 mg/kg of chloroquine. The diethyl ether extracts of *Eucalyptus camaldulensis* leaf shows suppressive activities on *Plasmodium berghei* in the animals treated. The group treated with standard drug (chloroquine) and the normal control group (uninfected untreated) survived beyond the experimental period, although the clearance time was faster for the standard drug. Changes in behavior and bodyweight were observed, this could be as a result of loss of appetite in the treated animal. Phytochemical screening revealed the presence of 6 phytochemicals including cardiac glycosides. The packed cell volume of the groups treated with diethyl ether extract of *Eucalyptus camaldulensis* decreased significantly as compared with both the positive and negative control groups while there is a slight increase in the normal control groups. From the result obtained, we can conclude that the diethyl ether leaf extract of *Eucalyptus camaldulensis* at 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of mice reduced parasitemia level. So diethyl ether fraction of *Eucalyptus camaldulensis* was effective in the therapeutic management of malaria parasite.

Keywords: Plasmodium berghei, Eucalyptus camaldulensis, diethyl ether, packed cell volume, parasitemial load, phytochemicals.

1. Introduction

Plasmodium falciparum, the pathogen most widespread human malaria, is becoming increasingly resistant to antimalarial drugs deal. This requires extra effort and continuous search for new drugs, especially with new modes of action (Muregi et al., 2003). In Sub-Saharan Africa, the proportion of patients utilizing antimalarial treatments outside the official circuit varies from 12 to 80 % (Bloland et al., 2000). Ethno medical and ethnobotanical studies now recognized to be the most viable methods of identifying new medicinal plants (Igoli et al., 2005). The use of medicinal plants plays an important role in daily health care in most rural area. Among some

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ethnic groups of Southern Cameroon like Pygmées-Baka, local medicine remains more popular than western medicine (Titanji et al., 2008). Traditional plants may supplement and/or even replace effective drug manufacturers, often inaccessible, for the treatment of malaria. Herbal medicine remains one of the common forms of therapy available for people worldwide.

Eucalyptus camaldulensis extract has many properties such as anti-oxidant, anti-blood proliferation, anti-cancer, anti-inflammatory, and anti-mold, painkiller anti-parasitic, anti-microbial, repellent and anti-virus properties.

Malaria life cycle involves two hosts and varies according to the type of plasmodium parasite that is causing the infection. There are slight variations in the life cycle of the plasmodium.

Therefore, aim of this research was to evaluate the *in vivo* antiplasmodial activity of diethyl ether extract of *Eucalyptus camaldulensis* leaf on malaria infection in experimental albino mice.

2. Materials and methods

Equipments

Conical flask, Bauchner funnel-flask, Filter paper, Cotton wool, Vacuum pump, Beakers, Evaporating dish, Mortar and pestle.

Reagents/Chemicals

Diethyl ether, Sulfuric acid, Chloroform, Benedict reagents, Hydrochloric acid, Meyer's reagent, Picric acid solution, Sodium hydroxide, Dragendorff's reagent, Ferric chloride reagent, Acetic anhydride, Water, Ammonia solution, Molisch reagent.

Materials for Infection of Experimental Mice

Methanol, Cotton wool, Normal saline, Razor blade, Butterfly needle, 2 ml syringe, Beaker, Slides, Giemsa stain, Microscope, Hematocrit centrifuge.

Preparation of Plant Material

The plant was collected from the University of Jos staff quarter. A freshly collected leaves of *Eucalyptus camaldulensis* was air dried at room temperature and the dried leaves were then pounded into its powdered form with the aid of mortar and pestle. Hundred grams of the plant leaves powder was weighed using a weighing balance into a 500 ml conical flask and was constituted with 300 ml absolute diethyl ether and was left to stand for 48 hours and properly mixed for 1hour. The content was filtered using a Whattman filter paper placed on a Buchner funnel-flask using a vacuum pump. The residue was rinsed several times and then filtered using fresh diethyl ether 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-plasmodia assay in experimental mice.

Preparation of Stock Solution

The stock solution was prepared just before use by dissolving the diethyl ether extract in 10% Dimethylsulfoxide (DMSO).

Preparation of Experimental Animal

Albino mice were obtained from the animal house, University of Jos with an ethical clearance. They were feed with a standard animal feed and cared for, for about three weeks before the infection in other to get a good weight. The experimental mice were separated into six groups with three mice in each group and labeled differently base on the difference in body weight. Highly infected bloods observed under the microscope were obtained from the tail of an infected rat. The body weight of each mouse was taken using a weighing balance before inoculation of parasite. About 3mls of blood from the infected mice was collected in a beaker and mixed with 0.2 ml of normal saline. The blood was injected into the experimental albino mice intraperitoneally. The infectious *Plasmodium berghei* parasite with a strain NK65 was obtained from malaria research institute, Ibadan. The uninfected mice were inoculated intraperitoneally with 0.5ml parasite suspension.

Experimental Design

The 18 mice were grouped into six (6) groups. Three treatment groups and three control groups (Positive, negative and normal control group). Each group has three mice.

The three treatments groups and the negative and positive control groups were infected with *Plasmodium berghei* parasite. The treatment groups were treated with 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of extracts. While the negative groups were left untreated.

Group A with 3 mice were infected and treated with 100 mg/kg of the extract per body weight per day.

Group B with 3 mice were infected and treated with 200 mg/kg of the extract per body weight per day.

Group C with 3 mice were also infected and treated with 300 mg/kg of the extract per body weight per day.

Group D (Positive control) with 3 mice were infected and treated with 25 mg/kg of standard drug (chloroquine) per day.

Group E (Negative control) with 3 mice were infected and untreated.

Group F (Normal control) with 3 mice were uninfected and untreated.

Inoculation of Parasite into Experimental Mice

The animals were inoculated using the method described by (Mann *et al.,* 2011). Infected mice were sacrificed and the blood sample was diluted with 0.2ml of normal saline, and the mixture was injected into the healthy mice intraperitoneally.

Administration of Plant Extract and Standard Drug (Chloroquine)

The mice that were infected with *Plasmodium berghei* were treated with diethyl ether extract of *eucalyptus camaldulensis* leaf intrapertoneally. And chloroquine was administered to the positive control group after 48 hours of inoculation daily for 10 days. The body weight of each mouse was taken using a weighing balance after administration.

Parasitemia Determination

Parasitemia was monitored by preparing a thin blood film. Two drops of blood collected from the tail of the mice were placed on one end of a labeled clean slide, then the edge of another slide is put in contact with the drop and the drop is allowed to bank evenly behind the speeded. Then the pusher slide is used to push the drop in smooth quick molten. The smear covers about half of the slide and air dry under a shade and dust free area. The film is then fixed with methanol for 2minutes and air dry, after then the fixed film is stained with giemsa stain and washed with clean water after about 10minutes. The number of parasites is then determined microscopically under x100 magnification with a drop of immersion oil placed at the tail end of the slide.

Packed Cell Volume (PCV) Determination

To determine the percentage of packed cell volume of blood sample, the blood sample is collected from the mice into an E.D.T.A container containing an anti-coagulant and a capillary tube were filled to two third with well mixed blood and one end of the tubes were sealed with plasticine. After that the filled blood was placed in the microhaematocrit centrifuge and spun at 12000 g for 5minutes and the spun tube is placed on a designed hematocrit reader and the PCV was read as percentage.

Packed cell volume (PCV) is the percentage of red blood cells in a circulating blood, when a known volume of blood is centrifuged at a constant speed for a constant time. A decreased in PCV generally means red blood cells loss from any variety of reasons like cell destruction, blood loss, and failure of bone marrow production. While an increased in PCV generally means dehydration or an abnormal increase in red blood cell production.

Phytochemical Analysis of the Plant Extract

Diethyl ether extract of *Eucalyptus camaldulensis leaf* 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, (1996) with slight modification.

Test for alkaloids

To 2 mls of extract, few drops of dragendorf's reagent were added to give orange colorations which indicated the presence of alkaloids.

Test for flavonoids

To 2 mls of the extract, few drops of 5 % lead acetate were added to give a cream light color which indicated the presence of flavonoids.

Test for tannins

To 2 mls of the extract, few drops of 10 % ferric chloride were added to give a deep bluish or greenish color which indicated the presence of tannins.

Test for Saponins

To 1ml of the extract, 4 mls of distilled water was added and shaken vigorously. Formation of froth indicated the presence of Saponins.

Test for terpens and steroids

To 1ml of the extract, 2mls of concentrated Sulphuric acid was added along-side of the test tube. Formation of reddish brown ring at the interphase indicated the presence of terpens and steroids.

Test for cardiac glycosides (Salkowski's test)

2 mls of the extract was dissolved in 2mls of chloroform and Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interphase indicated the presence of cardiac glycosides.

General test for balsam

3 drops of alcoholic ferric chloride were added to 2 mls of the extract. A dark green color formation indicated the presence of balsam.

Test for carbohydrates

5 drops of the extract were added to 2.0 mls of Benedict's reagent, placed on a hot plate for 5 minutes and was observed for the formation of brick red precipitation which indicated the presence of carbohydrates.

Test for phenol

To 2 mls of the extract, 2 mls of ferric chloride was added and observed for the formation of a deep bluish-green coloration which indicated the presence of phenol.

Test for resins

To 2 ml of the extract, 2 mls of acetic anhydride was added and drops of concentrated Sulphuric acid were added to observe for a violet color which indicated the presence of resins**.**

3. Results and discussion

Percentage Yield and Phytochemical Screening

The percentage yield of the Diethyl ether leaf extract of *Eucalyptus camaldulensis* was calculated to be 4.03 % and the result was given in Tables 1, 2 show the results obtained from the phytochemical screening of the Diethyl ether extract of powdered leaf of *Eucalyptus camaldulensis* and alkaloids, tannins, terpenes and steroids, balsam, resins, and cardiac glycosides were present.

Parasitaemia Count

Effect of Diethyl ether leaf extract of *Eucalyptus camaldulensis* on parasitaemia level of *Plasmodium berghei* infected experimental mice were shown in Figure 1. Generally, compared to the negative control group all treatment groups had lower parasitaemia. Conversely the positive group which was treated with 25 mg/kg of chloroquine had much lower parasitaemia compared to the other groups (the treatment groups and the negative control group which was infected and untreated). Over the experimental period the level of parasitaemia was in a constant increase in negative control group and a constant decrease in positive control group. Among the treatment groups there was more suppressive effect of the plant extract on the parasitaemia level, treated with 200 mg/kg body weight.

Packed Cell Volume

Effects of Diethyl ether leaf extract of *Eucalyptus camaldulensis* on packed cell volume of *Plasmodium berghei* infected experimental mice were shown in Figure 2. The packed cell volume of the groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg extracts, 25 mg/kg chloroquine and the group infected but untreated decreased by 19 %, 22 %, 18 %, 4 % and 12 % respectively. Whereas the group uninfected and untreated increased by 1 %.

Table 1. Percentage yield of the diethyl ether leaf extract of *Eucalyptus camaldulensis*

Eucalyptus camaldulensis	**Weight (g)**
Leaf extract	100.00
Diethyl ether extracts	4.03
Extract yield (%w/w)	4.03

Table 2. Phytochemical constituent of the diethyl ether extract of *Eucalytus camaldulensis* leaf

METABOLITE	**RESULT**
Alkaloid	+
Flavonoids	-
Tannins	+
Saponins	-
Terpenes and steroids	+
Cardiac glycosides	+
Balsam	+
Carbohydrate	-
Phenol	-
Resins	+

KEY
- = absent
+ = present

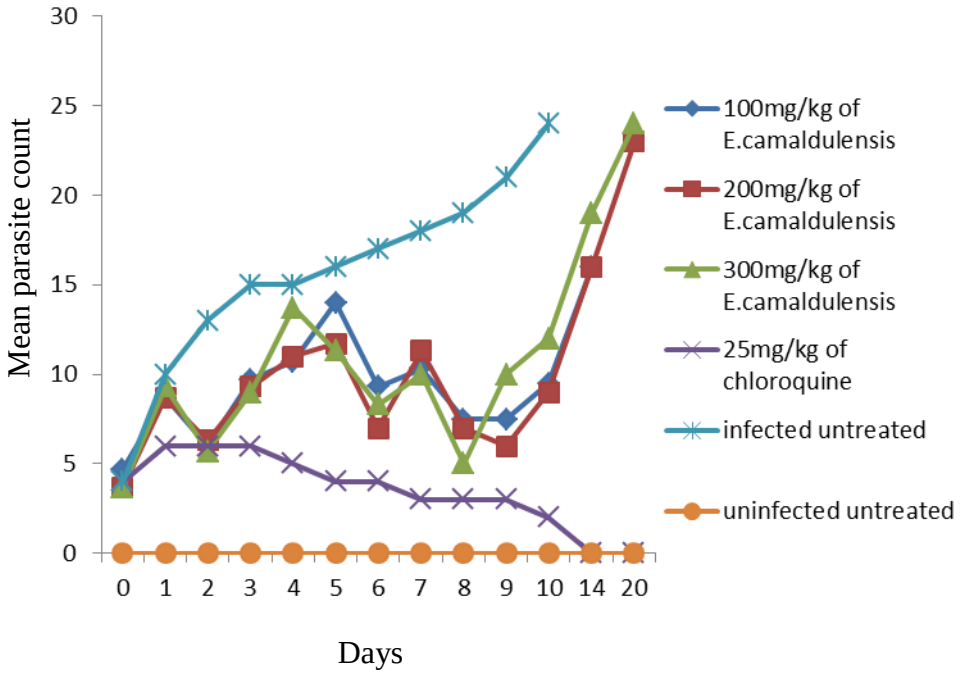

Fig. 1. Average mean parasitaemia count from day one of administration

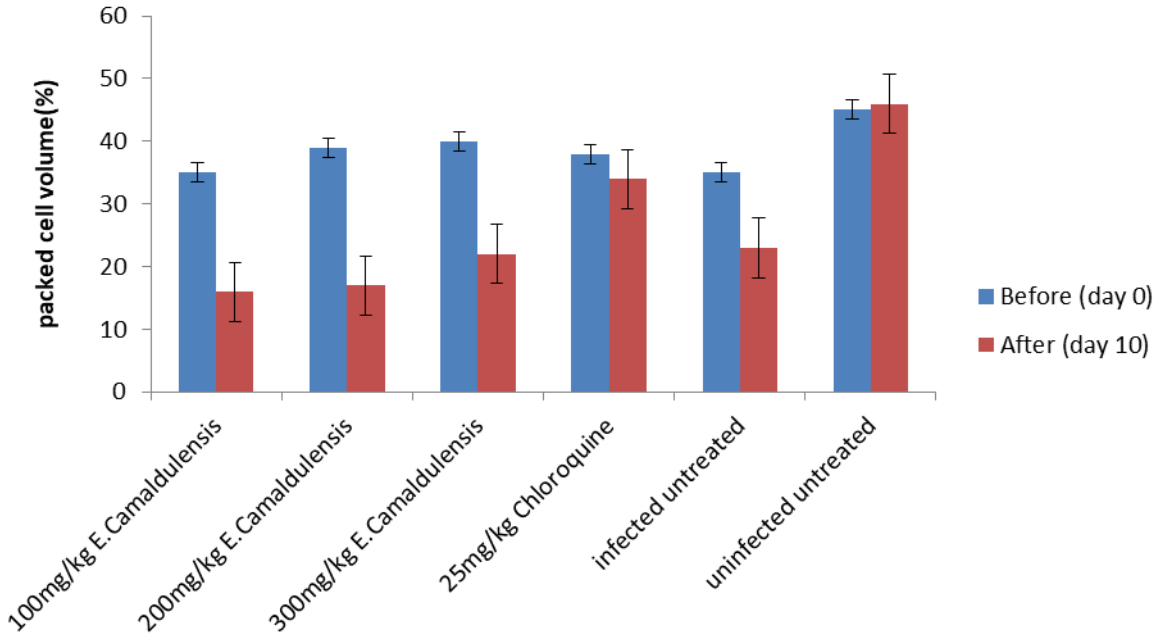

Fig. 2. The packed cell volume (PCV) of mice before inoculation of *Plasmodium berghei* and after administration

Differences in Body Weight

Effects of Diethyl ether leaf extract of *Eucalyptus camaldulensis* on the body weight of white albino mice infected with *Plasmodium berghei* were shown in Figure 3. The body weight of the groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg extract, 25 mg/kg chloroquine and the group infected but untreated reduced by 1.47 g, 4.16 g, 1.16 g, 2.12 g and 1.2 g respectively whereas, the group uninfected and untreated increased by 5.2 g.

Survival Time

The death rates of the experimental albino mice were monitored daily after the inoculation of *Plasmodium berghei* as shown in Table 3. The groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg and the group infected but untreated died on day 17, 25, 25 and 14 respectively whereas, the groups treated with 25 mg/kg chloroquine and uninfected and untreated group lived beyond the experimental period.

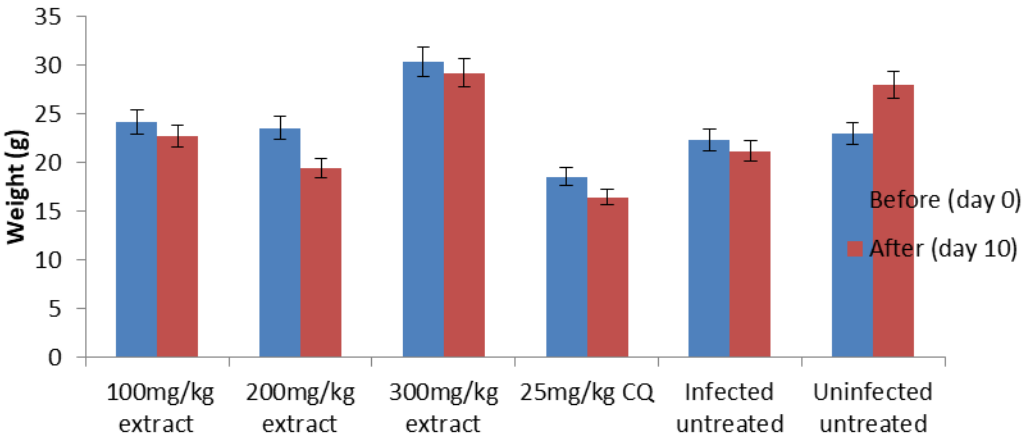

Fig. 3. Average mean weight of mice before inoculation of parasite and after administration

Table 3. Survival rate of experimental mice

GROUPS	DAYS OF SURVIVAL POST-INFECTION
A (100mg/kg extract)	1 7
B (200mg/kg extract)	2 5
C (300mg/kg extract)	2 5
D (25mg/kg CQ)	Was cured and Survived all through experimental period
E (Infected Untreated)	1 4
F (Uninfected untreated)	Survived all through experimental period

4. Conclusion

The percentage yield of the Diethyl ether leaf extract of *Eucalyptus camaldulensis* was calculated to be 4.03 % and the phytochemical screening of the Diethyl ether extract of powdered leaf of *Eucalyptus camaldulensis* are alkaloids, tannins, terpenes and steroids, balsam, resins, and cardiac glycosides. The body weight of the groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg extract, 25 mg/kg chloroquine and the group infected but untreated reduced by 1.47 g, 4.16 g, 1.16 g, 2.12 g and 1.2 g respectively whereas, the group uninfected and untreated increased by 5.2 g. The groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg and the group infected but untreated died on day 17, 25, 25 and 14 respectively whereas, the groups treated with 25 mg/kg chloroquine and uninfected and untreated lived beyond the experimental period.

The phytochemical screening of the crude diethyl ether leaf extract of *Eucalyptus camaldulensis* showed that the leaf contains useful phytochemicals which contributed to its anti-plasmodial activities in experimental mice. At the different concentrations of the diethyl ether extract 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 200 mg/kg concentrations, the parasite load was seen to reduce and at 100 mg/kg and 300 mg/kg concentrations, the parasite was seen to also reduce but not as the former. Indicating that if treatment period is extended and a higher dosage concentration is administered, the parasite will be cleared completely. *Plasmodium berghei* was used in the prediction of treatment outcome and hence it was an appropriate parasite for this study (Dikasso 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 ag ents obtained from plants are expected to prevent body weight loss in infected mice (Bantie et al., 2014). This research study present that the weight of the 200 mg/kg of the diethyl ether leaf extract of *Eucalyptus camaldulensis* significantly reduced with decrease in parasitaemia level when compared with the weight of the 100 mg/kg, 300 mg/kg and the group infected but untreated. While there is a significant increase in the weight of the group uninfected and untreated whereas, there is a decrease in the weight of the group treated with 25 mg/kg chloroquine.

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