Original Research Article
Journal der Pharmazie Forschung (Formerly-**Recent Advances in Pharmaceutical Science Research**)
Vol-2 | No-1 | 2013

Evaluation of murine model of malaria using ethanolic extracts of Pride of barbados *(Caesalpinia pulcherrima)*

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Abstract

In the present work, evaluation of in-vivo anti-malaria activity of ethanolic leaf extracts of pride of Barbados (*Caesalpinia Pulcherrima)* was investigated using rodent malaria parasite-*Plasmodium berghei*, and Swiss albino- mice as the experimental animal. The results showed that the extracts have 57% mean suppression action against the rodent malaria plasmodium berghei. This is indicative of the scientific evidence that ethanolic extracts of pride of Barbados (*Caesalpinia Pulcherrima*) posses moderate anti-malaria activity against a murine model of malaria.

Keywords: Ethanolic leaf extracts, *Caesalpinia pulcherrima*, plasmodium berghei, murine model of malaria

1.0 Introduction

Malaria is a parasitic infection transmitted by the bites of anopheles mosquitoes, infected with plasmodium species. There are two models of malaria- the murine model and human model. The murine model consists of plasmodium berghei. The human model consists of four species: namely plasmodium *falciparium*, plasmodium *vivax*, plasmodium *malariae*, and plasmodium *ovale*. In Nigeria, the human model is mostly caused by plasmodium falciparium and plasmodium *malariae*. The female anopheles mosquito transmits these parasites to humans.

Malaria as a disease primary affects poor populations in the tropical and subtropical areas, where the temperature and rainfall are sufficient for the growth of the vectors and parasites [1]. Malaria is a major global health problem. An estimated 247 million malaria cases were reported globally and nearly a million deaths annually [2]. Malaria is one of the major tropical parasitic diseases responsible for numerous morbidity and mortality among children, elderly and pregnant women [3].

In the south, eastern, and south-south parts of Nigeria, malaria parasites are transmitted all year round while in the northern part of Nigeria, it is mostly seasonal [4]. Orthodox drug resistant malaria has become a major problem in the malaria treatment. Resistance in vivo has been reported against almost all anti-malaria drugs except Artemisin and its derivatives [5]. This malaria drug resistance problem is the major trust for a search for an alternative drugs and hence the search for remedies in medicinal plants available in the local environments [6]. It is believed that if the herbs used to treat malaria by our forefathers in Africa centuries ago, were not effective, malaria would have destroyed the entire populations of Africa [7]. This is the basis for the in vivo screening of the leaves of pride of Barbados for its anti-malaria properties. This is the subject matter of this research work; the in vivo study of anti-malaria properties of ethanolic leaf extracts of pride of Barbados (*Caesalpinia Pulcherrima*) against Murine model of malaria parasite.

2.0 Materials and methods

Sample Collection: The leaves of pride of Barbados (*Caesalpinia Pulcherrima*) were harvested from the pride of Barbados plant grown within the Michael Okpara University of Agriculture, Umudike, Ikwuano Local Government Umuahia Abia State, Nigeria. The harvested leaves were identified and authenticated by a Botanist Dr. Garuba Omosun, of the Department of plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. A voucher specimen of the leaves was deposited at the University Herbarium.

Animal studies: A stock of thirty Swiss albino mice aged 4-5 weeks old were procured from University of Nigeria, Nsukka through the assistance of Dr. Ngongeh LA. of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The Swiss albino mice were maintained on standard mouse diets with clean water supplied and in the Animal House of the College of Natural and Applied Science until they are 6-8 weeks old when they were used for the experiments.

All chemical reagents used in this work area analytical grades from May and Baker chemicals Ltd., USA

Source of the murine malaria parasites: The murine malaria parasites- plasmodium

berghei, were obtained from the laboratory of Dr. A. O Aina of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. Five Swiss albino mice, aged 6 – 8 weeks old were infected by intra-peritioned injection of parasitized blood obtained from previously infected donor-mouse at NIMR. The infected mice were transported by road in a standard mouse plastic cage to Michael Okpara University of agriculture, Umudike. The infected mice were maintained on standard diets and water in the animal house. The mice were checked for the presence and level of *parasitaemia* after one week of acclimatization. The presence of the murine malaria parasites were confirmed by simple microbiological assays. The parasites were subsequently maintained by periodic passage and transfer into new sets of mice.

Sample preparation: The leaves of pride of Barbados harvested were air-dried at the laboratory room floor for two weeks. Thereafter the dried leaves of pride of Barbados were sieved into fine powder using 2mm- sieve. The powders were stored in a clean labeled air-tight container properly sealed until required for analysis.

Extraction of the active ingredient: One hundred gram of fine powdered leaves of pride of Barbados (Caesalpinia Pulcherrima) were weighed out and poured into a clean labeled out and poured ethanol was measured out and poured into the conical flask using what-man 40 filter paper. The filtrate known as the ethanolic extracts was evaporated to dryness using temperature regulated electric water bath. The dark brown leaf extracts obtained after the evaporation was stored in air-tight glass-desiccators until required for analysis.

All chemical reagents used in this work area analytical grades from May and Baker chemicals Ltd., USA.

Acute Toxicity Test: Three groups of the mice of both sexes were administered single oral doses of ethanolic leaf extracts of pride of Barbados (*Caesalpinia Pulcherrima*), 125mg, 250mg and 500mg 1kg body weight to determine the lethal dose at 50% (D_{50}) of the extracts. The extracts solution was first dissolved in ethanol and made up in distilled water. The control group received the mixture of distilled water and ethanol. The mice were put in plastic cages and observed for possible mortality and behavioral changes over a period of 24hr. the number of death occurring in the treated groups were used for measuring the lethal dose 50(LD_{50}) values.

Inoculation of *Plasmodium berghei* in Mice: Blood specimen was obtained from the snipped tail of infected mice to determine the percentage parasitaemia. Highly parasitized blood (≥ 4000 parasites/ML) was collected by retro-orbital procedure from the donor mouse on the day of inoculation of the groups (Day_0). The blood was diluted in phosphate buffed saline (PBS) to obtain inoculums of approximately 10^8 infected red blood cells per ml (RBCs/ml). Each mouse was then injected intra-peritionally with 0.1ml of diluted blood to deliver on inoculums of 10^7 parasitical RBCs/ml. The control mice were intra-peritionally injected with 0.1ml of sterile PBS

Evaluation of In vivo Anti-Malaria Activity: On day 0 (DO) after the inoculation of the test mice, the inoculated mice were divided into two groups. Each mouse in group1 was given by oral route 50mg/kg body weight of the ethanolic leaf extracts of pride of Barbados (*Caesalpinia Pulcherrima*) given 0.2ml of the solution. The treatment continued till day 4 (D_4). The mice in groups served as untreated control.

On day5 (D_5), blood specimen was taken from the cut tail of each mouse to determine the parasite level.

Preparation of thin-blood specimen [8]: A drop of blood was placed at one degree at one edge of a clean microspore shoe. The blood was spread out to make a thin blood smear. The blood smear was allowed to air dry. The smear was fixed by applying 1 – 2 drops of absolute methanol to the smear for approximately 3minutes. After air drying the thin blood smear was sterile with 10% giemsa solution for approximately 10 minutes. The four stained blood smear examined microscopically using X100 oil immersion objective. Four areas of the shoe containing approximately 250 RBCs per field were examined. The number of parasitized RBCs in the four fields was counted. The number of parasitized RBCs per 1000 RBCs was used to calculate the percentage parasitaemia.

3.0 Results and discussion

The results of the murine model of malaria studies using ethanolic leaf extracts of pride of Barbados (*Caesalpinia Pulcherrima*) are presented below.

Treated group	Ethanolic leaf extract 50mg/ml	Control untreated mice
M_1	17/1000	26/1000
M_2	14/1000	43/1000
M_3	19/1000	51/1000

Table-1: Estimation of parasitaemia of infected and treated mice.

From Table-1, the following calculations were carried out:

Mean parasitaemia for treated mice using 50mg/ml extracts = $\frac{14+19}{3} = \frac{16.67}{1000}$

Mean parasitaemia for control = $\frac{43+51}{3} = \frac{40}{1000}$

Percentage (%) parasitaemia for treated m ice 50mg/ml ethanolic leaf extracts.% = x = 1.667 = 1.7%

Percentage (%) parasitaemia for control = $100 = 4\%$

Average percentage suppression of the *Plasmodium berghei* in the infects mice using the established 50mg/ml of the ethanolic leaf extracts of pride of Barbados (*Caesalpinia Pulcherrima*) from the LD_{50} studies carried out is equal to percent (%) parasitaemia of untreated groups minus percent (%)of untreated divide the number of days of untreated group A. This is calculated thus as : $\frac{100}{1} = 57.5\%$

From the evaluation of the Murine model of malaria using infected Swiss albino mice infected by Murine malaria parasite, *plasmodium berghei* and treated using the established dose of 50mg/ml of the ethanolic leaf extracts of pride of Barbados (*Caesalpinia Pulcherrima*). From the Table-1, and he calculations, therefore the result obtained indicates that the ethanolic leaf extracts possesses moderates anti-malaria activities in a Murine model of malaria.

4.0 Conclusion

The study has established facility for evaluation of anti-malaria activity of the leaf

extracts of the medicinal plants pride of Barbados ((*Caesalpinia Pulcherrima)* using Swiss albino mice secondly, this work on the in vivo anti-malaria activity of pride of Barbados has established a scientific evidence to support moderate activity of this extracts in suppressing the *plasmodium berghei* in infected mice.

Acknowledgement

We sincerely appreciate the directorate of research and development (DRD) Michael Okpara University of Agriculture, Umudike and the tertiary education trust fund (TET FUND) Nigeria for the sponsorship of this project.

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