

## ***In vivo* evaluation of anti-malarial activity of stem and root extracts of *Erythrina abyssinica***

### **Abstract**

**Aims:** The aim of the study was to determine the *in vivo* anti-malarial activity of stem and root extracts of *E. abyssinica* using the 4-day suppressive *in vivo* anti-malarial test. **Methodology:** Female mice weighing approximately 20±2g were intra-peritoneally injected with mice passaged *Plasmodium berghei* parasites. The extracts were then administered orally 2h post-infection and, subsequently, daily for 4 days. On the 4<sup>th</sup> day, blood smears were prepared from all the mice, stained with giemsa and parasitaemia as well as chemosuppression determined.

**Results:** Comparatively, the root extracts exhibited higher chemosuppression than stem extracts and the level of chemosuppression was dose dependent being the highest at 50mg/kg and lowest at 12.5mg/kg. Survival time in extract treated and chloroquine treated groups was 2 to 3 fold higher than the -ve control.

**Conclusion:** These findings suggest that the root extracts are more efficacious in suppressing the development of full blown malaria compared to stem extracts and may be a useful candidate in managing malaria in future.

### **Introduction**

Malaria is a parasitic disease caused by plasmodium parasites transmitted through female anopheles mosquito bites. It causes up to 7 million mortalities per year globally and, in 2015 alone, it is estimated that it caused over 4 million deaths globally 92% of which were in sub-Saharan Africa, 8% in South-East Asia and 2% in the Mediterranean region [1]. Out of the over 4 million deaths reported in 2015, 70% were reported in children aged below 5 years. Therapeutic management of malaria has, over the years, been complicated by resistance of plasmodium parasites against conventional drugs. Consequently, this dilemma has ignited a surge in the search for alternative remedies against malaria parasites.

Several extracts of plant origin have been tested for their antimalarial activity with remarkably encouraging results [2, 3, 4, 5, 6, 7, 8, 9; 10, 11]. In this study, attention was focussed mainly on *Erythrina abyssinica*; a branched, deciduous shrub or tree belonging to the genus *Erythrina* with rounded, spreading crown growing up to 15 metres tall. The plant is widely distributed in Africa from Sudan, Ethiopia, Angola, Zimbabwe to Mozambique. Its bark is traditionally used to treat a wide range of ailments including malaria.

Previously, flavonoids and isoflavonoids with *in vitro* antiplasmodial activities have been isolated from the root [12] and stem [13] barks of *E. abyssinica*. Similarly, extracts from the root and stem barks of *E. sclouxii*, another species of the same genus, have also been shown to have *in vitro* antiplasmodial activity [14]. However, no *in vivo* studies have been undertaken to determine the efficacy of these *E. abyssinica* extracts on live animals. The purpose of this study was, therefore, to determine the antiplasmodial activity of these extracts on *in vivo* systems.

### **Materials and methods**

#### *Animal handling and care*

Forty five (45) female 6 weeks old Swiss mice weighing 20±2g were obtained from Kenya Medical Research Institute (Kemri) ([www.kemri.org](http://www.kemri.org)), housed in cages measuring approximately 45cm x 28cm x 21cm and maintained under hygienic conditions in a well ventilated room with feed and water

provided *ad libitum*. The animals were allowed one week acclimation before commencement of the study.

#### Donor mice infection

Five (5) of the original 45 animals were infected with stock blood containing *Plasmodium berghei* parasites and used as donors. In brief, frozen blood containing *P. berghei* parasites (stored at  $-80^{\circ}\text{C}$ ) was thawed and subsequently diluted in phosphate saline glucose (PSG) at the ratio of 1:1. The diluent was prepared by mixing phosphate saline glucose and glycerol at the ratio of 70:30. 200 $\mu\text{l}$  of the diluted heparinised blood containing *P. berghei* parasites were then injected intra-peritoneally into each of the five mice and parasitaemia monitored through blood smear until the 20% threshold was reached. These infected animals then served as donors to the experimental animals.

#### In vivo bioassay

The *in vivo* antimalarial bioassay was conducted using the four-day *in vivo* suppressive antimalarial test [15]. In brief, once a parasitaemia of 20% was achieved in the donor mice, they were euthanized using diethyl ether and *P. berghei* infected blood obtained by cardiac puncture. The blood was then diluted as before and randomly injected intra-peritoneally into the remaining forty female experimental mice.

Subsequently, the forty mice were randomly divided into eight (8) groups each comprising 5 animals. Groups 1, 2 and 3 were orally treated with root extracts dissolved in 1% DMSO at dosage rates of 50, 25 and 12.5mg/kg respectively while groups 4, 5 and 6 were orally administered the stem extracts dissolved in 1% DMSO at the same dosage rates respectively. Group 7 animals (+ve controls) were each orally administered a known antimalarial drug (chloroquine) at 10mg/kg while group 8 animals (-ve controls) were only given 1% DMSO (1ml/100g). Four (4) days post-infection, blood smears were prepared from each animal with blood obtained from the tail vein, fixed in methanol and stained with 10% giemsa. Chemosuppression for each test group was then determined by subtracting the parasitaemia of the test groups from the -ve control groups and expressing as a percentage of the -ve control [4, 11, 16].

#### Survival time

All the animals were maintained beyond the 4-day suppression period to determine their survival time. Survival time was determined by the duration the animals remained alive post-infection; the longer the survival time the stronger the suppressive effect.

#### Results

Root extracts of *E.abbyssinica* appeared to be more efficacious at dosage rates of 25 and 50mg/kg but less so at 12.5mg/kg as demonstrated by the percent chemosuppression (Table 1). The percent chemosuppression on *P. berghei* infection was as high as 77% in mice treated with root extracts at 50mg/kg, 71% in those treated with 25mg/kg and only 48% at 12.5mg/kg. Corresponding results using stem extracts were much lower at all doses suggesting that the stem extracts were less potent than the root extracts. The chloroquine treated group (+ve control) showed marginal chemosuppression at 6% tending closer to the -ve controls which had no drug intervention at all.

Survival time expressed as mean $\pm$ sem varied in the extract treated groups between 26 $\pm$ 9.0 and 38.4 $\pm$ 8.8 days in the 25mg/kg and 50mg/kg root extract treated respectively. This variation was, however, not significant at  $p\leq 0.05$ . Mice treated with 12.5 mg/kg of both root and stem extracts showed longer survival time compared to those treated with 25mg/kg of each respective extract. The survival time in all the extracts and chloroquine treated groups were generally higher than that of the -ve control.

**Table 1:** %chemosuppression by stem and root extracts of *E. abyssinica* and survival time (in days) in mice challenged *in vivo* with *P. berghei* parasites.

Groups	Dosage rates (mg/kg)	Parasitaemia (%)	Chemosuppression (%)	Survival time (days) (Mean±SEM, n=5)
Root extract treated	50	2.7	77	38.4±8.8
	25	3.4	71	26.0±9.0
	12.5	6.2	48	33.5±10.7
Stem extract treated	50	7.6	32	27.5±9.5
	25	11.0	8	38.0±8.9
	12.5	10.0	16	38.3±10.7
+ve control	10	11.2	6	25.3±11.8
-ve control	1% DMSO	11.9	0	13.0±3.0

### **Discussion**

Percent chemosuppression is regarded as an important indicator of the suppressive effects of the antimalarial agents in early infection by plasmodium parasites. Often, the percent chemosuppression  $\geq 50\%$  implies moderate to very good antiplasmodial activity on a scale where 100 to 90% suggests very good activity; 90 to 50% good to moderate activity; 50 to 10% moderate to weak activity and 0% generally inactive [9]. In the present study, the percent chemosuppression for the root extracts was well above 50% at 25mg/kg and 50mg/kg. Taken together, the percent chemosuppression achieved by the root extracts could well be classified as moderate to good at 12.5, 25 and 50mg/kg. On the other hand, the percent chemosuppression for the stem extracts was generally below 50% suggesting that the stem extracts were less effective compared to the root extracts.

*In vitro* studies have demonstrated that the root extracts of *E. abyssinica* [12] and *E. burtii* [17] have potent antiplasmodial activity. The present *in vivo* findings, where the root extracts showed moderate to good chemosuppression compared to the stem extracts, further reaffirms the results of the previous

*in vitro* studies. This underscores the likely significance of *E. abyssinica* as an antimalarial and opens the door wide for its possible application as an anti-malarial preparation in future. To this extent, it rates fairly well among other previously studied plant extracts. For example, *Nefang*, a polyherbal antimalarial extract of Cameroonian origin has also been shown to have remarkable *in vitro* [18] as well as *in vivo* [19] antiparasitic activity and, in addition, it exhibits strong antioxidant potency [20]. Further, root extracts of *Lecaniodiscus cupanioides* have also shown strong *in vivo* antimalarial tendency suggesting that it may be useful as an alternative antimalarial agent [21]. Different parts of plants contain varying concentrations of biologically active ingredients (22). Likewise, since previous studies have shown that root extracts of *E. abyssinica* have more activity than stem extracts [12] in *in vitro* systems and confirmed by the present *in vivo* studies, it implies that the root extracts of *E. abyssinica* have more concentrations of biologically active ingredients, hence, more potent compared to the stem extracts.

Survival time is a measure of the duration it takes before animals under the influence of chemotherapeutic agents die [23]. The longer it is, the more protective the agent is. In this regard, the survival time of the extract treated mice were generally longer than those of the -ve control suggesting that the extracts offered some form of protection to the treated animals in comparison to the non-treated ones.

### **Conclusion**

This study has demonstrated that extracts from *E. abyssinica* have some value in managing malaria and, in particular, the root extracts appear to be more potent than stem extracts

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