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 4th 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 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. In all cases, the animals exhibited anaemia, cyanosis of the liver and spleen, hepatomegaly and spleenomegaly which indicated the development of malaria.

Conclusion: These findings suggest that the root extracts are more efficacious in suppressing the development of full blown malaria compared to stem extracts. However, the suppressive effects of the extracts could not directly inhibit the manifestation of the overt pathological lesions associated with malaria.

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, 10]. 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. Reference N°[11] must be cited before N°[12].

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. scleuxii*, another species from 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) (Web site?), 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 $^{-}80^{o}C$) 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. 200µl of the diluted heparinised blood containing *P. berghei* parasites was then injected intra-peritoneally into each of the five mice and parasitaemia monitored through blood smear until 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.

Methods of evaluation of :

anaemia

hepatic cyanosis

splenic cyanosis

hepatomegaly

splenomegaly

Results

Root extracts of *E. abbysinica* 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±sem varied in the extract treated groups between 26±9.0 and 38.4±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 extract and chloroquine treated groups were generally higher than that of the –ve control.

In all groups (extract treated, +ve control and –ve control), the common macroscopic pathological lesions observed included anemia (anaemia) , hepatic and splenic cyanosis, hepatomegaly and splenomegaly

Show results about:

Methods of evaluation of :

anaemia

hepatic cyanosis

splenic cyanosis

hepatomegaly

splenomegaly

Table 1: %chemosuppression by stem and root extracts of *E. abbysinica 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	50	2.7	77	38.4±8.8
treated	25	3.4	71	26.0±9.0
	12.5	6.2	48	33.5±10.7
Stem extract	50	7.6	32	27.5±9.5
treated	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 on early infection by plasmodium parasites. Often, the percent chemosuppression ≥ 50% implies moderate to very good antiplasmodial activity [17] (precise the rates of moderate, good and very good chemosuppression respectively, from ≥ 50% to 100%). 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, good or very good at 12.5, 25 and 50mg/kg respectively. 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.

Without experiences using scientific methods, without results related to anaemia, hepatic and splenic cyanosis, hepatomegaly and splenomegaly, don't discuss about.

Anaemia, hepatomegaly and splenomegaly were observed in all groups suggesting that in all groups, the parasites established themselves and even led to the development of malaria. However, the intensity of the disease was somewhat suppressed in chloroquine and extract treated groups as evidenced by the survival time. In both these groups, the mice survived longer than the negative control animals. Anaemia, hepatomegaly and splenomegaly are readily demonstrable in acute malaria. Whereas anaemia is mainly occasioned by the destruction of red blood cells in circulation, hepatomegaly and splenomegaly are, to a large extent, caused by sinusoidal dilatation with minor contribution from reticulo-endothelial hyperplasia and hypertrophy [18, 19]. Cyanosis of the liver and spleen is commonly encountered in cases of malaria and, usually, it is associated with reduced oxygen content in blood indicative of arterial hypoxaemia [20]. In this study, the hepatic and splenic cyanosis was observed in all groups including the groups treated with the extracts. This perhaps implies an apparent lack of strong antiplasmodial effects of the extracts on mice leading to destruction of red blood cells, hence, hypoxia manifested as cyanosis.

Discussion about anaemia, hepatic and splenic cyanosis, hepatomegaly and splenomegaly, is so longer than antiplasmodial *in vivo* activities so that we can suppose that the results were about related parameters. To be able to deal with anaemia, hepatic and splenic cyanosis, hepatomegaly and splenomegaly, related methods and results must be mentioned.

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