



ANTIPLASMODIAL AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACT OF *Hyptis spicigera* (LABIATE).

Ishaya Kato Auta

Department of Biological Science, Kaduna State University, Kaduna, Nigeria.

Abstract: The extracts of *Hyptis spicigera* is known for its analgesic activity. In northern Nigeria, it is used for the treatment of fever as well as evil spirit related diseases and for repelling mosquitoes. The activity of ethanolic leaves extract of this plant on antiplasmodia and anti-inflammation has not been investigated. This study is aimed at assessing the anti-plasmodial and anti-inflammation in mice and rats. The study was carried out using rodent Plasmodia (*Plasmodium berghei*) in mice and egg albumin induced inflammation in rats. The LD₅₀ of the extract was established to be 1059± 0.51 mg/kg i.p in mice. The result showed that the extract exhibited sufficient antiplasmodial activity in the models used, giving a suppressive activity of 54.02 - 64-12% and exhibited a significant curative effect of 2.83 ± 1.33 to 2.67 ± 2.28. The extracts gave a significant anti-inflammatory value against egg albumin induced inflammation in Laboratory rats. The phytochemical analysis of the plant extract indicated the presence of Flavonoids, Saponin, Glycoside, Tannins and Phenols. The observed pharmacological activity might be the scientific basis for the folkloric use of the plant in treating fever attack.

Keywords: Antiplasmodial, anti-inflammatory, ethanolic extract, leaf, Oedema, *Hyptis spicigera*.

INTRODUCTION

There is an urgent need for new antimalarial drugs, because malaria parasites especially *Plasmodium falciparum*, have become resistant to chloroquine and to other clinically used drugs (Peters, 1987; Bjorkmann, 1999). Much effort has been made to explore the potential of natural products from plants which are used in traditional medicine (Phillipson and Wright, 1991). Until now, only artemisinin and its derivatives appear to be near to the stage of commercial availability (Klayrnan, 1989). There are many potential natural product candidates which are waiting to be examined as possible anti-malarial drugs (Francois *et al.*, 1992). One recently discovered group of interesting natural products, the naphthylisoquinoline alkaloids, occur in species of the Ancistrocladaceae and Dioneophyllaceae.

The resistance of *Plasmodium falciparum* to chloroquine and other commonly used anti-malarial has continued to pose a serious threat to the chemotherapy of malaria (Ndifor *et al.*, 1992). This unfortunate development is further complicated by the higher cost, greater toxicity and limited availability of alternative drugs to chloroquine (Root and Bjorkman, 1991). The continued search for other effective, safe and cheap anti-malarial agents thus becomes imperative in the face of these difficulties. The Scientific evaluation of relevant medicinal plant parts that are used in traditional medicine offer some hope for developing new agents that may be suitable for the treatment of malaria infection or associated pathological complications such as inflammatory processes and pain that stem from the destruction of red blood cells during a malaria attack.

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The acute toxicity profile, in vivo anti-plasmodial and anti-inflammatory activities of the ethanolic extract of the leaves of *H. spicigera* were evaluated using Swiss albino mice and rats.

MATERIALS AND METHODS

Preparation of Plant Material

The fresh leaves of *Hyptis spicigera* were collected from Tammah Nasarawa, in Nasarawa State, Nigeria and identified at the College of Forestry, Jos, Nigeria.

The leaves were air dried for two weeks and milled to a coarse powder. Two hundred grams of the powdered material were extracted using reflux Soxhlet extractor for 72 hours with ethanol. The appropriate extracts were obtained and used in the experiment.

EXPERIMENTAL ANIMALS

Wistar rats weighing between 180-200g and Swiss albino mice 15- 25g of either sex, maintained under standard condition at the animal facility centre of NIPRD, Abuja, were used for the experiment. The animals were fed with standard diet of livestock feed and had access to water,

ACUTE TOXICITY (LD₅₀)

The Intraperitoneal (I.P) acute toxicity LD₅₀ of the extract was evaluated in Swiss albino mice at doses of 10-1000mg/kg. The animals were observed for 24 hours and 1.1350 was evaluated according to the Corke method (1983).

ANTI PLASMODIAL ACTIVITY

The mice were prescreened by taking blood from the tip of the tail. Thick and thin smears were made and observed to be sure that there was no animal harbouring rodent parasite. (*Plasmodium spp*). The anti-plasmodial activity of the extract was assessed by suppressive and curative methods (Saidu *et al.*, 2000).

PREPARATION OF THE INOCULUM

A donor mouse infected with rodent malaria parasite *P. berghei* with parasitaemia level of about 20-30% was anaesthetized with chloroform and blood was collected through cardiac puncture with a sterile and apyrogenic disposable needle and syringe. The blood was diluted

with normal saline in such a way that 0.2ml of the blood contained approximately 1×10^7 infected red cells.

SUPPRESSIVE STUDIES

In this studies, treatment with the various dose of the extract commenced immediately after the mice had been inoculated, early infection as described by Peters (1970).

Twenty [20] Swiss albino

mice of either sex weighing between 15-25g were chosen and divided into four groups of five mice each. An inoculum of 0.2ml was given to each of the twenty clean mice intraperitoneally. The extract 100- 400mg/kg was administered once for four [4]. days starting from the first day (D₀) and continuing up to Day four [D₄] by the subcutaneous (S.C.) route. A parallel test was run using chloroquine (sigma) 5mg/kg in group 4 to serve as positive control. The fifth group served as control and was administered normal saline (N.S.). From D₁ to D₄, thick and thin films were made from the blood. The films were fixed with methanol and stained with 4% Giemsa at pH 7.2 for 7 hours and examined microscopically. The number of infected and uninfected red blood cells were counted and the mean infection determined.

STATISTICAL ANALYSIS

The results are expressed as mean (\pm SEM) and the significance of differences between control and treated groups were determined using students' t-test for Tables 1 and 2.

RESULTS

ANTI PLASMODIA/ACTIVITY SUPPRESSIVE TEST)

The suppressive activity of *H. spicigera* ethanolic extract against rodent mice *Plasmodium berghei* is shown in Table 1. The various doses 100mg/kg, 200mg/kg and 400mg/kg administered subcutaneously, apparently reduced the mean parasitaemia significantly to 60%

The result for the curative activity of *H. spicigera* ethanolic leaves reveals a decreased in parasitaemia levels at different concentration of the extract. The parasitaemia levels decreased is similar to that of standard control



groups. There is no significant difference between the treated and control group $P>0.05$ for all the test doses employed (Table 1).

Table 1: Effects of the ethanolic extract of *Hyptis spicigera* leave and chloroquine (CQ) on *Plasmodium berghei* in mice [suppressive].

Treatment	Dose (mg/kg/day)	Mean parasitaemia	Suppression %
Control (CQ)	5	4.00±2.35	67.76
Control (NS)	5	21.75±4.21	00.00
H.S	100	10.00±4.22	54.02
H.S	200	7.80±2.61	64.12
H.S	400	8.25±0.75	62.07

C.Q, Choloquine

N.S, Normal Saline

H.S, *Hyptis spicigera*

CURATIVE TEST

The same procedure was used for curative studies as was described for suppressive studies. The treatment with the extract and chloroquine, 100, 200, 400mg/kg and 5mg/kg respectively commenced on D0 and continued daily until D7. Thick and thin blood smears were made on D3 and D7. The slides were examined for parasite clearance. The increase or decrease in parrasitaemia and mean survival time recorded are shown in (Table 2).

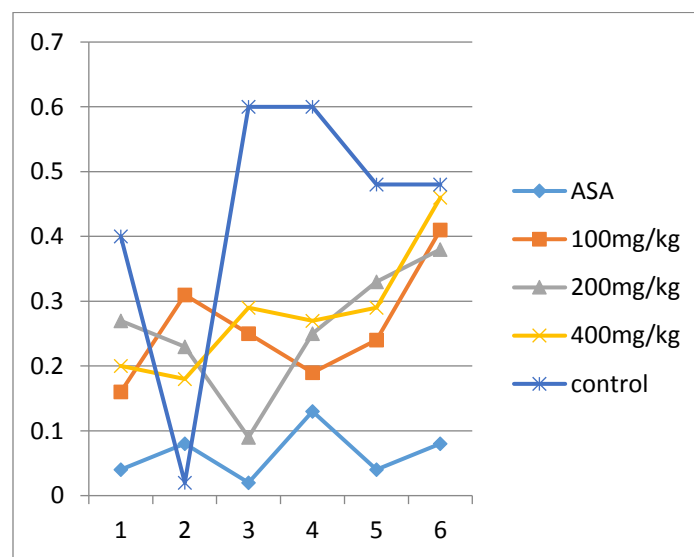
TABLE 2: Effect of the ethanolic extract of *Hyptis spicigera* leaves and chloroquine (CQ) on *Plasmodium berghei* in mice (Curative Studies)

Treatment	Dose [mg/kg]	Pre treatment D ₄	Post treatment D ₇
CONTROL	5	15.40±2.42	21.60±4.16
CQ	5	04.00±2.35	06.80±2.80
H.S	100	6.00±1.44	2.83±1.33
H.S	200	7.83±1.54	1.17±0.75
H.S	400	4.33±1.87	2.67±2.28

Anti-inflammatory activity

The effects of *Hyptis spicigera* ethanolic extract on egg albumin induced inflammation in rats shows that there is significant suppression of the inflammatory process $P<0.05$ at all dose levels tested when compared to the control with normal saline.

The Inflammation induced by injecting 0.1 ml egg albumin into the sub planter surface of the right hind paw for 30 minutes before intra-peritoneal administration of the extract showed that the extract possess some abilities to suppressed the Oedema of the treated wistar rats when compared with the negative and positive control. At 20 minutes, 100, 200 and 400mg/kg had lower values of the paw Oedema as seen in the positive control, the negative control values were higher as compared to the positive and treated group(Figure 1).



$P<0.05$

Figure 1: Anti-inflammatory activity of egg albumen induced Oedema in Wistar rat

treated with Ethanolic leaf extract of *Hyptis suaveolens*.

(ASA) = Acetylsalicylic acid

DISCUSSION



The Phytochemical results obtained from the plant reveals the presence of variety of active components which is an indicative of its high medicinal and therapeutic potentials. This is because these compounds formed the basis of the Pharmacologic effects of such plant, this is supported by the works of Haidet, 2003, Jigam *et al.*, 2004 who rank alkaloids the most efficient and therapeutically significant plant compound. The *in vivo* effect of the ethanolic extract of the leaves of *Hyptis spicigera* showed a significant suppression and curative of *Plasmodium berghei* in mice dosed with the plant extract as a curative or prophylactic agent. Most report on anti-malarial effects do not specify whether the plant acted on parasites or some other mechanisms. Such reports were also based mostly on *in vivo* studies (Valentine *et al.*, 1995; Taubi *et al.*, 1997; Acquaye *et al.*, 2001). It has been suggested that crude plant extracts tends to have better plasmodistatic than plasmodicidal effects because some unpurified bioactive principles may require initial conversions which time lag allows for parasite proliferation (Noedl *et al.*, 2003). More over the active components might not be present enough in the concentrations to effect rapid clearance of targeted organisms (Fidock *et al.*, 2004). The significant analgesic and Inflammation effects of *in vivo* of the ethanolic extract of *H. spicigera* leaves showed both analgesic and anti-inflammatory ($P < 0.05$). This may be due to the crude nature of the extract and possibly due to the presence of potentially antagonistic component in the extract whose activities may become manifest at a higher concentration. Plants with these added pharmacological phenomena in conjunction with antiplasmodial effects are better antimalarial than species with the later potential only (Pascual *et al.*, 2001).

There was no sharp difference between the effects of the high and low doses used in the study. The effects of the extract appeared to be comparable to as that of the standard dose of acetylsalicylic acid (ASA) at various concentrations of the extract, this suggest that the extract

contain reasonably potent analgesic and anti-inflammatory components.

The slight dose-dependent antiplasmodial activities in the study suggests the efficacy of *Hyptis spicigera* as antiplasmodial agent. This may be attributed to the presence of alkaloid, which has been reported by other authors to have effect on *Plasmodium* spp. (Webster, 1990; Omulokoli *et al.*, 1997).

The preliminary results from this study reveal that the aqueous extract of this plant has strong analgesic and anti-inflammatory properties which are vital for the management of clinical syndrome associated with malaria (Aguwa, 1996). This is in addition to its anti plasmodial effects.

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