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Research Article

Antimalarial Activity of *Acacia nilotica* Plant on Plasmodium berghei in mice

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Abstract: *The ethanolic bark extract of Acacia nilotica was investigated for antimalarial activity against Plasmodium berghei infected mice. The median lethal dose was determined to ascertain the safety of the extract in mice. Ethanolic bark extract of A. nilotica (100,250,500 mg/kg bw) was administered orally to Plasmodium berghei berghei mice in both the early and established phases. The antiplasmodial effect during early and established infection in addition to the repository action of at these doses caused 64.81-73.60% inhibition of parasitemia in the suppressive test and a mean survival time of 24 to 29 days representing 13.3 ± 1.63 to 7.6 ± 1.63 inhi bition of parasitemia in the curative test. These results show that the ethanolic bark extract of A. nilotica possesses significant antiplasmodial activity thus rationalizing its traditional use in malaria therapy.*

Keywords: *Acacia nilotica, Plasmodium berghei,* Antiplasmodial, Antimalarial.

INTRODUCTION

Malaria is one of the most important health problems in sub-tropical and tropical countries. The World Health Organization estimates that 2,300 million people or 41% of the total world population live in areas with malaria risk. More than 300 to 500 million clinical cases are reported annually resulting in at least 1.5 to 2.7 million deaths. Approximately one million deaths among children under 5 years old are attributed to malaria alone or in combination with other diseases[1, 2]. Mortality, currently estimated at about 781,000 people per year [3] is attributed to resistance of the parasite to commonly used antimalarial drugs. In addition to its direct health impact, malaria imposes a huge economic burden on afflicted individuals and nations, through high health care cost, missed days at work or school, and reduces economic output and productivity[4]. Currently, multi-drug resistance has become one of the most important problems

impeding malaria control efforts[5,6]. This has led to attempts to discover other antimalarial agents, mainly from plant sources medicinal plants may provide antimalarial drugs directly as in the case of quinine from cinchona bark or they may supply template molecules on which to base further new structure by organic synthesis from *Artemisia annua*. In Africa up to 80 percent of the populations still rely on herbal medicine to treat malaria and other diseases,[7] because of their affordability and accessibility. One of such popularly used medicinal plants is *A. nilotica*, a scented thorny three commonly found and used in Northern Nigeria as a traditional herbal remedy against malaria[8]. The fruits and root of the plant was reported to have antitubercular[9] and antidiabetic activities[10], while the bark is used in treatment of cough, diarrhea and as an aphrodisiac[11]. With this view, the present study was executed to analyze the antiplasmodial activity of the ethanol bark extract of *A. nilotica* in *Plasmodium berghei berghei* infected mice in order to provide scientific evidence for its continuous use in ethno therapeutic management of malaria.

MATERIAL AND METHOD

Plant material: *A. nilotica* bark collected from the local area of District Vidisha (M.P.) India. It was authenticated by the botany department of S.S.L.Jain P.G. College, Vidisha and was also confirmed by standard book. A voucher specimen is deposited in herbarium record at Pest Control and Ayurvedic Drug Research Laboratory, Vidisha (M.P.)

Preparation of plants extract: The bark was cleaned, air-dried at room temperature for 10 days and crushed into coarse power using pestle and mortar. The 225 gram of the powdered material was macerated with ethanol 350ml for 48h duration and up to 8 cycle of extraction in soxhelt apparatus. The crude extract were concentrated in a rotavapour below 40˚C after that, the crude extracts were evaporated on a water both to get dryness. The percentage of yield was calculated to be 8.16%. This ethanol extract was used in the all studies with doses expressed in mg / kg body weight of the animal.

Preliminary Phytochemical analysis: Phytochemical screening of the *A. nilotica* extract was carried out using standers procedure to test the presence of alkaloids, saponins, glycosides, flavanoids and tannins[12].

***In Vivo* Antimalarial Screening of Crude Extract:** Swiss albino mice of either sex weight between 25-30g were obtained from Bharat Animal House Jahagorabad, Bhopal (M.P.) India. Animal were maintained to standard laboratory condition, allowed to get acclimatized to a standard pellet diet, Golden Feed Pvt. Ltd., New Delhi and water ad libitum. Room temperature maintained at 25 ± 5˚C with 12 h light and dark cycle. In this study a total of 30 mice were randomly assigned into three treatment group and two control group (negative and positive control) with six mice in each group. All animal experiments were conducted according to the ethical approved by CPCSEA and Institutional Animal Ethics Committee (IAEC) guidelines. (Approval No. 804/03/CPCSEA).

Parasite inoculation: The *Plasmodium berghei berghei* NK65 Chloroquine- sensitive strain was obtained from Malaria Parasite Bank, National Institute of Malaria Research Center, New Delhi. The Albino mice previously infected with *P. berghei* having variable parasitaemia were used as donors. Parasitize erythrocytes were obtained from a donor infected mouse by cardiac puncture with a sterile needle and blood was collected in a petri dish with the help of anticoagulant (heparin). The blood was then diluted with normal saline (0.9%) based on the parasitaemia of the donor mice in such a way that 1 ml blood 5x107 infected erythrocytes. Each mouse received 0.2 ml diluted blood containing 1x107 *P. berghei* infected erythrocytes by intra-peritoneal rout. To avoid variability in parasitemia, the blood collected from all donor mice was pooled together, and the parasite was maintained by weekly passage to other mice. Protocol was described by Peters *et al.*[13].

Test on early malaria infection (4-day suppressive test): A modified peters 4-day suppressive test against *P. berghei* infected mice was employed[14]. On the fifth day (post day 4 treatments) blood was collected from the tail of each mouse the thin films fixed with methanol, stained with JSB I & II were prepared and

examined microscopically[15]. The parasitemia was determined by counting minimum of three fields per slide with 100 RBC per field[16]. The percentage suppression of parasitaemia was calculated for each test concentration by comparing the parasitaemia in infected controls with those received different concentrations of the extract. Percent parasitemia and percent parasitemia inhibition (%PI) were calculated using the modified[17].

Percentage inhibition of the parasite was calculated by the following formula.

$$\%PI = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in study group}}{\text{Parasitemia in negative control}} \times 100$$

Test on established infection (Curative Test): The inoculation and treatment procedure used for curative test were similar to the 4-day suppressive test described above except that in the curative test, treatment started on day three after infection was established. The treatment continued daily until day seven when thin blood films were calculated, stained and examined for parasite suppression. After the seven day, the mice were further observed for 30 days. Any death that occurred during this period was recorded and used to determine the mean survival time.

$$MST = \frac{\text{Total number of mice in that group}}{\text{Sum of survival time of all mice in a group (days)}}$$

Acute Toxicity Test: The oral acute toxicity of the ethanol extract was estimated in albino mice (25-30g) by medium lethal dose (LD_{50}) described by Lorke's method[18]. Three groups of three mice each were used and the animal was given extracts at doses of , 400, 800, 1200 mg/kg body weight respectively. Extracts were used dissolved in distil water. The animals were monitored for 24h and number of death per group recorded. The mice were observed for gross behavioral changes such feeding, hair erection, lacrimation, mortality and other sings of toxicity manifestation[19]. The mice have free access to food and clean water during the experiment.

Data Analysis: The results were expressed as mean ± SEM. The data was analyzed using student t-test and differences between mean were considered significant of ($P< 0.05$) level was used.

RESULT

The phytochemical screening of ethanolic extract of bark of *A. nilotica* revealed the presence of alkaloid, flavanoids and saponins. The ethanolic extract exhibit an LD_{50} above 800 mg/kg having shown no mortality at from 100 to 800 doses tested. The extract is assumed to be safe. Invariably, the experimental doses used were relatively safe. Early malaria infection or peters for day's suppressive utility test for the ethanol bark extract of *A. nilotica* produce a dose dependent suppressive activity was shown in the **Table-1**. The highest suppression of parasitemia was observed at dose of 500 mg/kg body weight of mice. Percentage suppression was observed to increase as extract conc. increased. After 4 days treatment with different extract doses, the mean parasitaemia of test group ranged from 9±1.09 to 12±1.09 while the corresponding value of the negative control group being 34.1±1.83. The mice treated with CQ were 3.3 ± 1.03 from parasites on 4th day. The antimalarial activity produced by the extract was statistically

significant when related to control. The result of the *in vivo* evaluation of the *A. nilotica* extract on the established infection showed a slight increase in suppressive activity. The extract was marginally active at mean survival time of 30 day as compared to 24 ± 1.09, 28 ± 1.83 and 29 ± 1.22 observed with 100, 250 and 500 mg/kg doses respectively **Table (2).** The mice that received 5mg/kg bw of Chloroquine per day, however showed the value 29.8 ± 0.40 suppression.

Table-1: Antiplasmodial activity of *A. nilotica* during Chemosuppressive Test

Extract	Dose (mg/kg)	Parasitemia count	% Inhibition
Saline Water	20ml	34.1 ± 1.83	-
Chloroquine	5	3.3 ± 1.03	90.32%
A. nilotica	*100*	12 ± 1.09	64.81%
	250	10.6 ± 1.03	68.91%
	500	9 ± 1.09	73.60%

Table-2: Antiplasmodial activity of *A. nilotica* during Curative Test

Extract	Dose (mg/kg)	Parasitemia count	Mean Survival Time (day)
Saline Water	20ml	39.8 ± 1.32	12.3 ± 1.50
Chloroquine	5	4.3 ± 1.03	29.8 ± 0.40
A. nilotica	*100*	13.3 ± 1.63	24 ± 1.09
	250	9.5 ± 1.87	28 ± 1.83
	500	7.6 ± 1.63	29 ± 1.22

Data are expressed as mean ± SEM

DISCUSSION

The extract of *Dodonaea angustifolia* contains different secondary metabolites that have antiplasmodial activity in other plants[20]. Tannins[21] and alkaloids[22], which have been suggested to be responsible for antiplasmodial activity of other plant, were also detected in the bark of *D. angustifolia*. As the antiplasmodial activity observed in many plants[23] was considered to result from single or combined action of metabolites, same could be said for the present study. Extract of D. angustifolia bark showed antimalarial activity against *P. berghei* infection in mice as evidenced by percentage of parasite inhibition. As shown from the result of the *in vivo* antiplasmodial studies. The effect on parasitaemia in this study are similar to the one reported by previous study such as on *Aspurus africanus*[24]. However, relatively higher

antiplasmodial activities than the present results have been reported on Nigella sativa and *Azadirachya indica*[25]. The extracts prolonged the mean survival time of the study mice indicating that the extract suppressed *P. berghei* and reduced the overall pathologic effect of the parasite on the study mice. However, neither the extracts nor the standard drug crude the infection. This could be due to recrudescence of *P. berghei* parasite after apparent cure. Antimalarial activity of *V. hastate* leaf extract might be attributed to the presence of phytochemical constituents such as terpenes and flavonoids implicated in antiplasmodial activities of many plants. However, the active compound(s) known to give this observed activity need to be identified. Some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation.[26] or by inhibiting protein synthesis[27] depending on their phytochemical constituents. The antiplasmodial properties of the extract and its fractions were investigated using standard models. It was found that reduced the parasitaemia in prophylactic, suppressive and creative models in a dose dependent fashion. Some secondary metabolites of plants have been reported to have antiplasmodial activity. Among these metabolites are alkaloids, flavanoids and triterpenoid such as limonoids and quassinoids[28]. Moreover, the leaf extracts of *A. indica* (abr. Juss) (IC_{50} 47.20 µg/ml) and *C. roseus* L (IC_{50} 49.63 µg/ml) showed good antiplasmodial activity. This may be due to the presence of alkaloids.[[29]] Agbedahunsi, 2000; have employed *P. berghei berghei* in predicting treatment outcome of suspected antimalarial agents, because of its high sensitivity to Chloroquine, making it appropriate for this study[30].

The results obtained from the present work support the traditional use of *A. nilotica* against malaria and confirmed the antimalarial activity of the plant. This plant bark is potential source of molecule for the development of compound as alternative antimalarial drug. Novel antimalarial compound can also be isolated from the most active fraction of *A. nilotica* and tested against human malaria parasite. However, further studies have to be conducted on its antiplasmodial activity as well as safety point of view.

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