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Research article

EVALUATION OF ANTIPLASMODIAL EFFECTS OF THE ETHANOLIC LEAF EXTRACT OF SPONDIA MOMBIN ON PLASMODIUM BERGHEI INFECTED MICE

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ABSTRACT

Malaria is a disease condition most peculiar to Sub-Saharan Africa and till today remains a challenge as a result of the resistance of the protozoa, Plasmodium species to currently existing drugs. Herbal medicines have been used in treating this disease for years and these medicinal plants have been sources of some antimalarial chemotherapeutic agents. Objectives: To evaluate the in vivo antiplasmodial effect of Spondia mombin leaf in mice infected with chloroquine-sensitive Plasmodium berghei, with a view to finding scientific evidence for the use of mixed water alcohol decoction of the leaf as traditional antimalarial remedy in Nigeria. Methods: Sixty healthy Swiss mice were used for the study. The 4-day suppressive test and curative effect against established rodent malaria infection in mice were analyed. The ethanol leaf extract preparation of S. mombin, 50 mg/kg, 100 mg/kg, 200 mg/kg and 10 mL/kg of distilled water for no drug control, chloroquine 10 mg/kg for standard control were administered orally to Plasmodium berghei infected mice. Results: The ethanol leaf extract showed that parasitaemia was dose dependently and significantly ($P < 0.01$) lowered in early infection, 80 – 92 % and established infection test representing 72-89% inhibition of parasitaemia, with a mean survival time of 23 – 28 days. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, steroids and terpenoids. The acute toxicity test was greater than 5000 mg/kg in mice. Conclusion: These study show that ethanol leaf extract demonstrated high antiplasmodial activity in a dose-dependent fashion. Thus, supporting claims of the plant's traditional therapeutic importance for malaria treatment by local population, and can be developed as an alternative therapy against the disease.

KEYWORDS

Spondia mombin; leaf extract; antiplasmodial; suppressive; curative

Introduction

Malaria is a widespread parasitic infection ravaging different parts of the world, especially within tropical and subtropical regions[1]. It is estimated that malaria infects more than 300 million humans every year, throughout the world, causing over 2 million hardships and death in children annually[1]. More than 40 % world's population resides in a malarious environment, most especially in Sub-Saharan Africa, that favours the growth of mosquitoes[2]. The most devastating manifestations of malaria in man are linked to Plasmodium *falciparum*. The parasite has resisted virtually all antimalarial drugs in use, making resistant strains spread fast[3]. Resistance to these agents has become a major challenge confronting its control in developing countries. Over past decades, attention generated by drug-resistant *Plasmodium falciparum* strains has led to growing interest in research for antimalarial remedies. Natural products from plants have maintained an important source of new agents against malaria[4]. Therefore, there is an urgent need to thoroughly examine these natural habitats from different communities possibly via discoveries which might be useful to mankind[5]. However, in traditional medicine, different plants and herbs have been prescribed as

an intervention for malaria conditions. Thus, studies on plants together with their beneficial effects could provide leads, for the synthesis of essential active metabolites.

S. mombin is an herbaceous plant species in the Fabaceae family, commonly found in tropical and subtropical regions. This plant, often referred to as "one-leaved clover," is well known for its medicinal uses and ecological significance. Native to Africa and Asia, *S.mombin* has spread to many other parts of the world due to its ability to thrive in a variety of soil types and climat. Its leaves, in particular, have garnered attention for their phytochemical and pharmacological properties[6], which make them useful in traditional medicine and potential modern therapeutic applications[7]. In traditional medical practice, the entire plant was administered to patients suffering from cytotoxicity, renal calculi, and sepsis [8]. The extract from the leaves has been tried for treating eye problems and earaches[9]. In cases of renal disease, skin issues, leprosy, and respiratory difficulties, the roots of this plant are used as a diuretic[10]. A cough may be treated by drinking a decoction made from the roots of the plant. The root of this plant is often employed in the treatment of renal and urinary tract disorders, as well as leprosy and pulmonary conditions. It is possible that *A. vaginalis* has a very significant role in reducing and avoiding hepatotoxicity caused by Necrotic Body[11]. For medical purposes, the whole plant is utilized and it is effective in curing bone fractures and sword cuts.

Methodology

Plant Material Collection and Authentication

The fresh leaves of *Alysicarpus vaginalis* was sourced from a local farmland in Umudim Amichi in Nnewi South Local Government area of Anambra State, Nigeria. A taxonomist in the Botany Department, Nnamdi Azikiwe University Awka, identified the plant part with a maintained voucher specimen Number 256[A] deposited in the herbarium. The leaves were washed in distilled water, air-dried for 7 day at laboratory temperature, and pulverized to fine powder. Four hundred (400) grams of the fine powder was soaked in 2.5 L ethanol and left for 48 h with intermittent agitation. The mixture was then filtered and the filtrate evaporated to dryness at reduced pressure and controlled temperature of 40 ^{o}C on a water bath. The semi-solid green powder (10.5 % ^{W}/w) obtained was transferred to a dry sample container, and then kept in a refrigerator until use.

Phytochemical analysis

The ethanol leaf extract of A. *vaginalis* was subjected to phytochemical analysis to determine the secondary metabolites following the procedures of Oloyede [12].

Animals used in the experiment

Male and female Albino mice (20–22 g) obtained from Animal House of the Department of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The male Albino mice were separated from the female and maintained under standard laboratory conditions of 25 – 30 ^{o} C, 12-hour light and 12-hour darkness cycles. They were maintained in clean cages with sawdust, which was replaced every two days and had access to a pellet diet and water *ad libitum*. The mice were maintained following the National Institute of Health Guide for Care and Use of Laboratory Animals[13]. This study was also conducted according to ethical guidelines on laboratory animal use and care in compliance to Animal Ethical Committee Guidelines of Faculty of Basic Clinical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State (FBCS/14/VOL.10/2024/055). Every effort was made to minimize animal suffering. These animals were randomly distributed into experimental groups.

Determination of acute toxicity test

Acute toxicity (LD50) of the extract was determined using Lorke's method 14. This study was in two phases and the mice used were deprived of food overnight before extract administration. In phase1, three groups of three animals per group were used and were orally given the extract in increasing doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. The mice treated were monitored for 24 h for signs of toxicity and mortality. With the absence of death after 24 h, phase 2 was introduced. Four groups of one

mouse were each given the extract orally at doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg and 10 mL/kg of distilled water. The animals were then observed for signs of toxicity and mortality for 24 h and 48 h respectively for late toxicity.

Plasmodium berghei

Plasmodium berghei NK-65 sensitive chloroquine infected mice was sourced from National Institute for Medical Research (NIMR), Lagos. They were kept in Animals House unit, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus. Parasites in mice were maintained by an intraperitoneal passage from mouse to mouse[15].

Inoculums

Infected red blood cells from the donor mouse were collected through cardiac puncture after anaesthesia with halothane using a sterile needle and syringe. Microscopic examination of smeared and stained thin blood film was used to establish parasitaemia. Each mouse was intraperitoneally injected with 0.2 mL containing 1×10^7 parasitized (*P. berghei*) erythrocytes suspension.

Suppressive study

A 4-day suppressive test[16] was employed for this experiment. Male and female (30) mice, 18 to 20 g were passaged with standard *P. berghei*. Infected mice were divided into five groups of six each in three test groups and received 100 mg/kg, 200 mg/kg and 400 mg//kg respectively, of the ethanol leaf. The drug-free infected control group was given 20 mL/kg of distilled water. While the standard control group was treated with Chloroquine, 10 mg/kg. The treatment was orally administered as a single daily dose for 4 days. On day 5, thin films of all mice in different groups were smeared from the tail of each mouse on a slide. The films were fixed and stained using 10 % Giemsa in pH 7.2 phosphate buffer, and the parasites' presence was determined in 1000 erythrocytes in 10 different fields under the microscope (CX 21, Olympus Corporation, Tokyo, Japan) [17].

Curative assessment

The study was performed using the method of Chandel and Bagai[18], Peters and Robinson[19], with a slight modification. In this study, treatment commenced 72 h post parasite inoculation and lasted for 4 days. *P berghei* infected non-treatment group was given distilled water (10 mL/kg). Then, test groups 2 - 4 administered doses of the ethanol root at 100 mg/kg, 200 mg/kg and 400 mg/kg. Whereas, reference drug control (group 5) was given 10 mg/kg chloroquine (Sigma chemicals). Thin films were prepared and viewed under oil immersion and the presence of parasites was determined as previously described. The Percentage reduction in parasitaemia was calculated using the formula below: % Reduction in Parasitemia = [(parasitaemia of parasite control – parasitaemia of extract treated) /parasitaemia of parasite control] x 100.

Statistical procedure

Values are expressed as means ± SEM. The data were analyzed with SPSS version 20 by one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test. A difference in the mean, $P<0.05$ was considered significant.

Results and discussion

Phytochemical test

Phytochemical screening of the ethanol leaf extract of *A. vaginalis* revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, steroidsand terpenoids, while anthraquinone and phlobatannins were not detected.

Acute toxicity test

No mortality was seen, following 5000 mg/kg administration of leaf extract of *Alysicarpus vaginalis*. Therefore, doses of 100 mg/kg, 200 mg/kg and 400 mg/kg from the extract, were experimentally within a safe margin.

Suppressive response

The extract showed suppressive activity in a dosedependent fashion. Doses used exhibited 80%, 87% and 91% Chemosuppression of parasitaemia, respectively. The activity of the extract was significant (P<0.01) when compared with the control. Chloroquine, the standard agent also caused 92% inhibition which was significant (P<0.01) compared to control (Table 1).

Curative response

In table 2, ethanol extract significantly (P<0.05 and P<0.01) exhibited a dose-dependent decrease in parasitaemia in the extract treated groups and a significantly (P<0.01) decrease in the chloroquine treated group.

In mean survival time (MST), there was a consistent increase in parasite density of the untreated parasite group. Mortality was witnessed in untreated on the 7th day and by day 10, all mice in the infected nontreated group died. But the extract-treated mice survived above 22 days. More so, few mice in 400 mg/kg of the extract survived up to a 30-day observation period. However, chloroquine treatment recorded no mortality (Table 3).

Table 1. Suppressive effect of ethanol leaf extract of S. mombin in mice infected from Plasmodium berghei

Treatment	Dose (mg/kg)	Mean Parasitaemia	% Suppression
Control	10 mL/kg	31.40 ± 0.30	-
S. mombin	50	6.40 ± 0.50	80b
S. mombin	100	4.20 ± 0.30	87b
S. mombin	200	2.80 ± 0.50	91b
Chloroquine	10	2.40 ± 0.30	92b

Values are expressed as mean ± SEM (n=6); a significantly different from control at P < 0.05; b significantly different from control at P < 0.01.

Table 2. Curative effect of ethanol leaf extract of S. mombin in mice infected from Plasmodium berghei

Treatment	Dose (mg/kg)	Parasite density (D3)	Parasite density (D7)	% Inhibition of Parasitaemia
Control	10 mL/kg	32.10 ± 0.50	41.70 ± 0.34	-
S. mombin	50	29.75 ± 0.60	11.80 ± 0.60	72a
S. mombin	100	29.40 ± 0.70	9.80 ± 0.30	77a
S. mombin	200	30.00 ± 0.60	7.50 ± 0.30	82b
Chloroquine	10	29.80 ± 0.60	4.80 ± 0.45	89b

Values are expressed as mean ± SEM (n=6); a significantly different from control at P < 0.05; b significantly different from control at P < 0.01; D3 = Day three, D7 = Day seven.

Table 3. Survival time of mice receiving different doses of ethanol leaf extract of S. mombin and chloroquine

Treatment	Dose (mg/kg)	Survival time (day)
Control	20 mL/kg	10.15 ± 1.11
S. mombin	50	23.50 ± 1.84a
S. mombin	100	25.00 ± 1.59a
S. mombin	200	28.65 ± 0.83b
Chloroquine	10	30.00 ± 0.00b

Values are expressed as mean ± SEM (n=6); a significantly different from control at P < 0.05; b significantly different from control at P < 0.01.

Discussion

Malaria infection is one of the most prevalent diseases in the tropical region with increased mortality and morbidity.the recent rise of *plasmodium* species resistance to orthodox drugs, there is an urgent need for

more exploration of medicinal plants for newer antimalarial agents. These plants could be attractive starting materials as they are found greatly in our environment. More so, a large population in countries with malaria as one of the prevalent diseases uses these herbs for their curative activities [20]. While orthodox formulations dominate research, as attention has now shifted to studies on natural products [21]. The present study employed *in vivo* antiplasmodial model in rodents since the model takes into account the possible involvement of the immune system and possible prodrug effect in the activity of the plants against the parasites [22]. Moreover, several antimalarial drugs such as artemisinin derivatives and chloroquine have been identified using in vivo model [23]. Both curative and suppressive models were employed in order to establish the curative capability on established infection as well as the schizonticidal activity of the plants. Antimalarial agents are expected to decreased parasitaemia and its symptoms. This can be achieved through various means like reducing parasite nutrient intake and interfering with the pathways such as the heme pathway [24] or they could negatively affect the growth and reproduction of parasites [25].

The extract exhibited high antiplasmodial action against the parasites at all the concentrations used during the 4-day early infection study. It also demonstrated high suppression of parasites comparable to chloroquine. The extract showed a dose-dependent activity, as dose-increased antiplasmodial level also significantly improved. There was a significant reduction in parasite density in the infected treated when compared to their infected but untreated counterpart, indicating the antimalarial property of this leaf extract. The observation supports its traditional application as an herbal remedy for malaria treatment. The extract showed an appreciable decrease in parasitaemia level at all doses in established.

The extract reduced parasitaemia in both the curative and suppressive models and made some of the mice survive for a longer time. Besides, the prolongation of the surviving times of the infected mice by the extract is a further proof of the strong antimalarial potential of the plants. The observed antimalarial activity of *S. mombin* leaf extract could be attributed to their phytochemical constituents including alkaloids, flavonoids, saponins, tannins, steroids, or glycosides either acting singly or synergistically as observed from the phytochemical analysis.

Various studies have supported the antimalarial activity of these plant constituents. Alkaloids and flavonoids have been reported to possess a broad spectrum of bioactivities including antimalarial activity[26]. Many alkaloids, in particular, possess antimalarial activity and this is exemplified by quinine which is one of the most important and oldest antimalarial drugs[27]. Other phytoconstituents including saponins, steroids, flavonoids[28], and terpenoids[29] have been reported to exhibit potent antimalarial activity. These previous reports are further support for the antimalarial activity of *S. mombin* since these phytochemicals were identified in these studied plants.

Furthermore, the *Plasmodium berghei* strain used in this study has been chloroquine sensitive. It has been reported that this agent disrupts the parasite's cell membrane and induces their auto digestion via the formation of FP-chloroquine complex which impaired heme polymerization [30]. Though the drug elicited marked suppressive and curative activities by decreasing the level of parasitaemia, the extract also exerted comparable antiplasmodial results, especially at the highest concentration. Moreover, there was no proof of toxicity in mice exposed to 5000 mg/ kg of the extract. Therefore, the extract can be taken as non-toxic in the acute studies. This good safety profile may explain why a large population of Nigerians use this mixture in malaria treatment.

Conclusion

The study have shown that *S. mombins* ethanol leaf extract possesses potent activity against malaria. It, may serve as affordable sources of drugs against the disease. The underlying mechanism associated with the antiplasmodial properties of the extract is being screened in our laboratory. However, separation and quantification of various active principles, aimed at identifying the isolates which confer its antiplasmodial properties will also be determined.

Declarations

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