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Research Article

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Antiplasmodial activity of the ethanol leaf extract of *Andrographis paniculata* (acanthaceae)

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Abstract *Andrographis paniculata* is a plant that grows well under shade. It is found in China, India and Nigeria, The plant is used traditionally in Nigeria to treat boils, swellings and pains. The Ethanol Leaf Extract of Andrographispaniculata was evaluated for its antiplasmodial activity using Suppressive, prophylactic and curative models in 120 albino mice. The plasmodium specie was *Berghei berghei*. A dose of 946.69 mg/kg/day of the extract reducedparasitaemia significantly ($^{b}P<0.01$) from21.33 ±1.02 to 12.51±1.12 and suppressed malaria by 43.74%. Artesunate, a standard anti-malarial drug (positive control) produced 56.25 % chemosuppression. The normal saline (negative control) did not suppress malaria. The Lethal dose (LD_{50}) of the extract was 316.23 mg/kg. The result of the phytochemical screening showed that the.extract contained tanins, polyphenols, terpenes, alkaloids, saponins and cardiac glycosides in abundance. These suggest that *Andrographis paniculata* leaf extract is active against *plasmodium berghei berghei* and by extension the plant can be used in the treatment of malaria. The phytochemicals are believed to be responsible for the anti-malarial activity.

Keywords Antiplasmodial activity, Ethanol leaf extract, *Andrographis paniculata.*

Introduction

In Africa about 300 million people are infected annually by the parasite *plasmodium falciparum*. Over one million deaths have been recorded in children less than five year [1]. Malaria may cause abortion and low birth weight in pregnancy. It leads to low mental and manual productivity. The diseases a major cause of poverty in Nigeria. About 46% of an average household income s expended on treatment of malaria [2-3].

Plasmodium resistance is the ability of the parasite strain to survive and or multiply despite the administration of a drug given in doses equal or higher than those usually recommended but within the limits of tolerance of the subject [3-4]. *Andrographis Paniculata* (Acanthaceae) is a medicinal plant used traditionally in Nigeria to treat several diseases including malaria [5-8].

This study intends to carry out the phytochemical screening and find out the bioactive constituents of the plant, carry out the acute toxicity study find the dose of the plant extract that is safe for consumption.

The study will also carry out the antiplasmodial activity of the extract to see if the scientific data generated would support the ethnomedical use of the plant in malaria treatment.

Materials and Methods

Ethical Approval: Approval for animal studies was obtained from the animal ethics committee of the College of Health Science University of Uyo, Ngeria.

Collection and Identification of Plant: The leaves of *Andrographis paniculata*was collected from Ikot Ide Village, in ObotAkara Local Government Area of AkwaIbom State, Nigeria in October, 2015. The leaves were authenticated by Dr. (Mrs) M. E. Bassey a taxonomist in the department of Botany and Ecological Studies, University of Uyo. The leaves were shade dried for 14 days to reduce moisture.

Extraction: The dried plant material was pounded to form powder. The powder (810g) was macerated with N-hexane for 72 hours at room temperature (27 ^{o}C±2) with shaking two times daily to enhance the extraction process.

The extract was filtered using filter paper (WatmanNo.1). The filtrate was evaporated to dryness at room temperature, covered and preserved in the refrigerator at -4^{o}C until required for use.
The residue obtained was macerated with 70% ethanol. The same procedure was repeated. The ethanol extract gave a better yield of 17.05g (2.10 %) and it was then used for the study.
Phytochemical Screening: The ethanol extract was screened phytochemically to ascertain the bioactive constituents present in the extract. The standard method was employed (9).
Breeding of Animals: One hundred and twenty (120) albino mice weighing between 15-20g were collected from the animal house, Department of Pharmacology and Toxicology, University of Uyo, Nigeria. Food and water were provided *ad libitum* for the mice during breeding. The saw dust in the animal cage was changed two times a week. The animal house was cleaned and disinfected regularly.
Microorganism: *Plasmodium berghei berghei* (ANKA,chloroquine sensitive strain) was obtained from the National Institute of Medical Research (NIMER) Lagos. The parasite was maintained by sub passage in mice.
Parasite Inoculation: 0.3ml of infected blood containing about 1 x 10^{7}plasmodium *berghei berghei* parasitized erythrocytes was used to inoculate each mouse intraperitoneally. The inoculum contained 5 x 107 plasmodium *berghei berghei* parasitized erythrocytes per ml(4).
Preparation of Inoculum: The inoculum was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in preparations indicated by both determinations: Red blood counts and percentage parasitaemia (10).
Animal Grouping: The mice were placed in twenty cages of 6 mice each making a total of 120 animals. Thirty (30) mice were used for acute toxicity study and 90 for antiplasmodial study. These animals were given the extract, distilled water (negative control) and artesunate (positive control). The administration was done orally and oral canula was employed.
Group A mice received 10ml/kg of distilled water.
Group B mice received 316.23mg/kg body weight of extract.
Group C mice received 632.46mg/kg body weight of extract.
Group D mice received 948.69mg/kg body weight of extract.
Group E mice received 5.0mg/kg body weight of Artesunate.
The treatment was done for three days consecutively (D_o, D_1 and D_2) between 8.0 AM and 9.0 AM. On the fourth day (D_3), the mice were inoculated with *plasmodium berghei berghei*. The level of the parasitaemia was determined by blood smears, seventy-two hours later.
Stock Concentration of the Extract: This was prepared by dissolving 1.0g of the extract in 10ml of distilled water. The required concentrations were prepared from the stock by serial dilution to obtain low, middle and high dose of the extract.
Acute toxicity test (LD_{50}): A total of thirty (30) albino mice weighing 15-20g were used for the toxicity test. The animals were fasted for 24 hours, weighed and divided into five groups. Each group of mice was given different different doses of the extract intraperitoneally based on their body weight and then observed for physical signs of toxicity for 24 hours [11-12].
Evaluation of Antiplasmodial activity of the leaf Extract of *Adndrographis Panculata* :
Evaluation of the Prophylactic (Repository) Activities of the Ethanol Leaf Extract:
The prophylactic activity of the extract was determined using the method described by Peters (1965). Thirty (30) mice were divided at random into five groups. Each group contained six mice. The drug was administered to the mice using oral cannula for three days. On the anaesthesized using chloroform, Blood was collected from the heart by cardiac puncture with disposable needle and syringe. The mice were then passage with 0.2ml of the parasite intraperitoneally. A control test was run using artesunate (5.0mg/kg) and normal saline (10.0ml/kg).
The percentage parasitaemia on the slides were determined by staining with Leishman's stain and viewed under the microscope.

Evaluation of suppressive activity of the ethanol extract (4 – day test):
The suppressive (Schizontocidal) Activity of the extract, distilled water and Artesunate against early *P. berghei berghei* infection in mice was evaluated [13].
Another thirty (30) mice were divided at random into five groups of six mice each. On the first day (D_o) the mice were infected with the parasites from the donor mouse by sub passage. These animals were then given the extracts, distilled water and Artesunate orally using oral cannula. On the fourth day, blood was taken from the tail and smeared on the slides. The slides were viewed under the microscope and percentage parasitaemia counted.

Evaluation of Curative activities of Ethanol extract (Rane's test)

The schizonticidal activity of the extract and artesunate in established infection was evaluated using Rane's test [14]. The mice (30) were inoculated with plasmodium *berghei berghei* and left for 72 hours to develop malaria infection. This was followed by treatment with Extract, Artesunate and Normal saline. Thin blood film was made from tail blood on the fifth day. Staining of the film was done with Leshman'sstain to reveal parasitized erythrocytes out of 500 in a random field of the microscope. The average percentage suppressive effect of parastaemia was calculated in comparison with the control as shown below.

$$\frac{\text{Average \% parasitaemia in negative control} - \text{Average \% prasitaemia in positive in positive control}}{\text{Average \% parasite in negative control}}$$

Data Evaluation and Statistical Analysis

Data obtained from this work were analyzed statistically using student's t-test and a probability level of less than 5% and 1% were considered significant.

The Mean Survival Time (MST)

The Mean Survival Time (MST) of the mice in each treatment group was determined over a period of 29 day (Do –D28).

$$\text{MST} = \frac{\text{No. of days survived}}{\text{Total No. of days}} \text{X100}$$

Results

Table 1: Result of the Phytochemical screening of the ethanol leaf extract of *Andrographis paniculata*

Constituents	Concentration
Resin	-
Phlobatanin	-
Tannins	+ + +
Ploy phenols	+ + +
Carbohydrates	+ +
Terpenes	+ + +
Alkaloids	+ + +
Balsams	-
Flavonoids	+ + +
Saponins	+ + +
Anthraquinones	+
Cyanogenic glycoside	-
Cardiac glycoside	+ + +

Key: + + + = present in high concentration
+ + = present in high moderate concentration
+ = present in high low concentration
- = absent

Table 2: Results of Repository (prophylactic) activity of the ethanol leaf extract of *Andrographis paniculata*on *Plasmodium berghei berghei.*

Treatment	Group	Dose(mg/kg)	Parasitacmia	% Chemosuppression
Distilled water	A	10ml/kg	24.33±0.88	-
Extract	B	316.23	22.66±0.72	6.86
	C	632.46	21.40±0.94[a]	10.97
	D	946.69	14.23±1.55[b]	42.45
Artesunate	E	5.0	8.6±0.57	67.11

Values are expressed as mean ± SEM significance relative to control
[a]P< 0.05, [b]P< 0.01, n = 6

Table 3: Suppressive (4-days test) Activity of the Ethanol extract of Andrograhis Paniculata on plasmodium bargheiberghei in mice.

Treatment	Group	Dose(mg/kg)	Parasitaemia	% Chemosuppression
Distilled water	A	10ml/kg	21.33±1.02	-
Extract	B	316.23	18.04±1.15	15.61
	C	632.46	14.33±0.77[a]	32.81
	D	946.69	12.51±1.12[b]	43.74
Artesunate	E	5.0	9.44±0.63[c]	56.25

Values are expressed as mean ± SEM significance relative to control; [a]P< 0.05, [b]P< 0.01, n = 6

Table 4: Curative Activity of Ethanol Leaf Extract of *Andrographis paniculata* on *Plasmodium berghei berghei* in mice on day seven (7)

Treatment	Group	Dose	Average parasitaemia	%chemosuppression
Control (ml/kg)	A	10	26.66± 2.33	-
Crude extract	B	316.23	11.33±1.52	58.4±1.05
(mg/kg)	C	632.46	6.43±2.02[a]	76.2±1.56
Crude	D	948.69	5.0±0.57[b]	81.3±1.44
Arterunate	E	5.0	3.00±0.63	89.7±1.29

Values are expressed as mean ± SD significance relative to control.
[a]P< 0.05, [b]P< 0.01, n = 6

Table 5: Result of Mean survival time of mice treated with Ethanol Leaf Extract of *Andrographis paniculata* during established *Plasmodium berghei berghei* infection in mice.

Treatment	Group	Does(mg/kg)	Mean Survival Time (Days)
Distilled water	A	10ml/kg	11.33±0.43
Extract	B	316.23	13.28±1.20
	C	632.46	15.56±0.75[a]
	D	948.69	18.47±0.60[b]
Artesunate	E	5.0	19.33±0.53

Values are expressed as mean ± SEM. Significance relative to control
[a]P< 0.05, [b]P< 0.01, n = 6

Discussion

Andrographis paniculata (Acanthaceae) is a medicinal plant traditionally used in Nigeria totreat boils, swellings, skin diseases, frequent urination, diarrhea and malaria (15). The several uses of this plant in foklure medicine prompted the scientific evaluation of its antiplasmodial activity invivo (1). The result obtained has justified the traditional uses of this plant.

The result of this study shows that as the dose of the ethanol extract of *Andrographis paniculata* increasd, the level of parasitaemia decreased in the prophylactic, suppressive and curative models examined.

Terpenes and alkaloids were present in abundance in the extract. These compounds are believed to be responsible for the antiplasmodial activity. The result is in agreement with an already reported study (16). In the said study, some secondary metabolites of plant such as alkaloids and terpenes have been found to exhibit antiplasmodial activity. Monoterpene indole alkaloids have been involved in the endoperoxidation by generating high doses of free radicals. These free radicals destroy the cell membrane of the plasmodium parasite and consequently kill it (17).

Conclusion

In Nigeria, the leaf decoction of *Andrographs paniculata* is drunk for prevention and cure of malaria. The scientific data generated by this study support the ethnomedical use of the plant in malaria treament. Further study to isolate, identify and characterize the active principle responsible for the antimalarial activity is recommended.

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