DETERMINATION OF THE ACTIVITY OF AFRICAN MISTLE TOE (*Tapinanthus dodoneifolius)* EXTRACTS AGAINST *Plasmodium berghei* IN INFECTED RATS

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Abstract—This *In vivo* study was aimed at determining the antiplasmodial activity of Ethanol and Acetone extracts of *Tapinanthus dodoneifolius* (African mistletoe) against Chloroquine sensitive NK47 *Plasmodium berghei* in infected female swiss albino mice. The plant leaves were extracted using soxhlet apparatus and extracts subjected to phytochemical screening using standard procedures. The lethal dose (LD_{50}) of the extracts was determined using Lorke's Method. The antiplasmodial activity was determined by evaluating the percentage chemosupression. The extracts were found to contain Secondary metabolites. All extracts was found to be non-toxic to the experimental animals and possess antiplasmodial activiies of 98.4% and 98.1% for the Acetone and ethanol extracts respectively.

Index Terms— African Mistletoe, Antiplasmodial activity, infected Rats, *Plasmodium bergei*, Secondary Metabolites

1 INTRODUCTION

PLANTS have been a source of medicine in the past centuries and today, scientist and general public recognize their value as a source of new or complimentary medicinal product [23].

Medicinal plants constituents are an effective source of both traditional and modern medicines; Herbal preparations form the basis for many therapeutic drugs and they are the first line of treatment for 80% of the world's population due to their availability and also because they are relatively inexpensive [11].

In West Africa, mistletoes are found on many tree crops of economic importance including the Parkia tree (*Parkiabiglobosa*), shea butter tree (*Vitellariaparadoxa* Gaertn) and many others [8, 20, 12, 7]

The ethnomedicinal uses of mistletoes have been in the hands of very few herbal practitioners who claimed a general use to counter sorcery and magical powers, to treat health conditions such as sterility, problems associated with urino-genital system, rheumatism and also mental health. Mistletoes of the Loranthaceae and Viscaceae, are widely used in various cultures around the world to treat various ailments including hypertension, cancer, and diabetes, or used as a diuretic agent [1, 13]. For example, the tea made from Loranthaceae spp. is believed to cure bone fracture and body pain [13]. Remedies for tumour in Southwestern Ethiopia were reported to be prepared by crushing fresh leaves of *Tapinanthus globiferus* and mixed with cold water to be administered orally. In the Ebolowa region of Cameroon, one handful of the fresh leaves of *Tapinanthus globiferus* is usually mixed with one handful of the root bark of *Boswella odorata*, the ingredients are macerated in 5L of local beer and one glassful is taken twice a day for two weeks to cure syphilis [6]

In Saudi Arabia, fresh stems of *Tapinanthus globiferus* (local name, *Hadhal*) are given orally to all types of livestock for the treatment of fever and removal of placenta after parturition.

The first Rodent malaria *Plasmodium berghei* was identified and isolated from wild rodents in central Africa by Ignace Vince and Marcel parasite Lips in 1948 and subsequently adapted to mice, rats, Hamsters and Gerbils, and easily maintained laboratory bred mosquitoes such as *Anopheles stephansi*,which has since been used in malaria studies chemotherapy, and has also serverd as surrogate model of Human Malaria in the field of immunology, genetics, molecular biology and biochemistry [4].

Plasmodium berghei is used as a model for Human malaria parasite. It is similar in structure and gene content with the human malaria parasite [6]. *Plasmodium berghei* is being used due to its ability to infect rodents and relative ease of genetic engineering. The organism's genome can be modified using biotechnology as compared to *Plasmodium falciparum* and *Plasmo-*

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dium vivax [10]

MATERIALS AND METHODS

3.1 Collection of Plant Material

Collection of plant material was carried out by harversting the leaves of *Tapinanthus dodoneifolius* from branches of the host plant *Parkia biglobosa*. This was carried out during the hot season in the month of May in Bassawa, Zaria Local Government Area of Kaduna State.

Collection of the leaves was done by hand picking. Hands were covered with hand gloves and the plant material was collected and transported in a sterile polythene bag to reduce microbial contamination.

3.2 Authentication

The plant materials and host plant were authenticated at the Herbarium of the Department of Botany, Ahmadu Bello University, Zaria and identified as belonging to the Family Loranthaceae, Species: *Tapinanthus dodoneifolius* with voucher number 1175.

The Host plant was identified as belonging to family Mimosaceae: Fabaceae, species: *Parkia biglobosa* with Voucher number 2846.

Processing of Plant Material

The leaves were handpicked cleaned, and dried in a shade to reduce moisture content, prevent enzymatic and microbial activity and also extend shelf life.

Choice of Solvents

Acetone: Manufactured by Tedia Company Inc. 1000 Tedia way, Fairfield, 04 45014 USA.

Ethanol: Merck KGa A,64271 Darmstadt Germany,EMD Millipore Corporation. 290 Concord Road,Billeria MA 81821.

Soxhlet Extraction

One thousand grams (1000g) of the plant material was placed in the thimble, which was made of thick filter paper and was placed in the main chamber of the soxhlet extractor.

The solvent was placed in the round bottom flask and sufficient heat was added to evaporate the solvent which was then cooled by the condenser. The chamber containing the plant material was slowly filled with the warm condensed liquid which at a level was automatically emptiedby a siphon side arm, with the solvent running back down to the distillation flask. After so many rounds, the solvent became concentrated as described by [10].

After extraction, the extracted substance was placed in a ceramic bowl and placed on a water bath. The solvent was evaporated yielding only the extracted compound.

The percentage yield for the two extracts was calculated using a formula demonstrated by [5].

A sample of the extract was collected aseptically into glass Jars.

Acute toxicity testing for the extract was carried out using Lorke's Method.

Experimental Animals for Malaria Studies

Healthy female 7-8 week old Swiss Albino mice weighing 13-27g were used for the malaria studies. Animals were weighed before inoculation and prior to treatment. Animals were allowed to acclamatise for 24 hours at room temperature and were kept at room temperature housed in standard shoe box cages. Animals were fed with pelleted commercial feed and treated water freely. Animals groups were distinguished by markings made at the tail. Room was adequately lit and ventilated.

Table 1: Experimental Design

S/NO	Groups	Description	Treatment
1.	A	Normal Control	No infection, No treatment
2.	B	Experimental control	5mg/Kg Chloroquine
3.	C	Acetone Extract I	1000mg/Kg
4.	D	Acetone Extract II	500mg/Kg
5.	E	Acetone Extract III	250mg/Kg
6.	F	Ethanol Extract I	1000mg/Kg
7.	G	Ethanol Extract II	500mg/Kg
8.	H	Ethanol Extract III	250mg/Kg
9.	I	Negative Control	Infected, No Treatment

Activity of the Extract on Established Infection (Curative test)

The inoculation and treatment procedures used for curative test was done according to the modified Peter's Curative test [21] as described by [25]. The Animals were injected interperitoneally with Standard inoculums of Chloroquine Sensitive *Plasmodium berghei* infected erythrocytes using a sterile 1ml syringe. The normal control group was left out.

Infection was allowed to establish for 72hours. Group A, normal control group was neither inoculated nor treated. Group B was the experimental control. Animals were inoculated with 0.2ml parasitised erythrocytes and treated with 5mg/ml Chloroquine Phosphate injection. Group C (inoculated with approximately 0.2mls of parasitized erythrocytes and treated with 1000mg/kg Acetone extract), Group D (inoculated with approximately 0.2mls of parasitized erythrocytes and treated with 500mg/kg Acetone extract), Group E (inoculated with approximately 0.2mls of parasitized erythrocytes and treated with 250mg/kg Acetone extract), Group F (inoculated with approximately 0.2mls of parasitized erythrocytes and treated with 1000mg/kg Ethanol extract), Group G (inoculated with approximately 0.2mls of parasitized erythrocytes and treated with 500mg/kg Ethanol extract). Group H (inoculated with approximately 0.2mls of parasitized erythrocytes and treated with 250mg/kg Ethanol extract) and group I (inoculated with approximately 0.2mls of parasitized erythrocytes and no

treatment given).
Thin blood films were made from the tail of each mouse After 72 hours of infection and after the 72 hours treatment.
The extracts and Chloroquine were administered concurrently twice daily before feeding the animals. The animals were kept for 28 days post innoculation and the mean survival time for each group was determined.
Mean survival time= Total days of survival of each animal in the group
Total number of animals in the group

Collection of Blood Samples
Blood was collected from the tail.The tail was cut 1mm from the tip of the tail using a scapel blade [24]. The tail was gently stroked with the thumb and finger to enhance blood flow. The first few drops were wiped off with a disinfected cotton wool while subsequent drops of blood was smeared directly on a glass slides and thin films were made [16].

Staining and Microscopy
Staining was carried out using commercially prepared Field Stain. Thin blood films were allowed to dry for about an hour. The films were stained with field stain. The slides were then air dried and viewed under the microscope [16].

Stock Preparation and Dosage Reconstitution
Stock solution was prepared by dissolving in appropriate volume of solvent. Vehicle used was normal saline and the dosage was reconstituted in appropriate volumes by serial dilution procedures not exceeding 1mg/ml as recommended by [17, 14].

Innoculum Preparation
Parasites were obtained from National Institute for Medical Research Lagos (NIMR) and maintained at the Animal House of Pharmacology Department of Ahmadu Bello University by Passaging. A donor mouse was infected intra-peritoneally and kept for five days to allow for parasitaemia to build up to about 25% -30%.A mature 6 week old mouse has about $5x10^8$ RBC's/1ml.Therefore a 1:5 dilution gave $1x\ 10^8$ RBC's/ml. 0.2ml of this dilution delivered approximately $1x10^7$ parasitised RBC's [9].

Route of Administration
The route of administration for the extract was oral via an intubation cannula as it is the route of delivery that projects the route of administration to humans, while the parasitized erythrocytes were administered intra-peritoneally.

Parasitaemia Count
A minimum of of three (3) fields of view of the microscope and a total of parasitized and non-parasitized erythrocytes were counted and subsequently the percentage infected cells for each group were determined. Results were be expressed based on percentage parasitaemia (average) for the three different fields.

Data Analysis
The data of the study will be expressed as mean SEM standard error of mean for each group.
The data on parasitaemia and mean survival time was analysed using SPSS version 20.0. The differences between the means of measured parameters were compared using one way ANOVA followed by Duncan's Multiple Comparison Test. The p values<0.05 were regarded as statistically significant.

RESULTS

Plant Preparation/Extraction
A quantity of 1000g was weighed out from the dried and pounded leaves of the leaves of *Tapinanthus dodoneifolius*. The ethanol extract yielded a green viscous liquid extract while the acetone extract yielded a solid gummy extract with a percentage yield of 300ml and 30g respectively.

Acute Toxicity Testing
In phase 1 of the experiment, 100% of the experimental animals survived. Similarly in phase two there was also 100% survival for the Ethanol and Acetone Extracts of *Tapinanthus dodoneifolius.* There were no signs of toxicity observed in both phase 1 and Phase 2 of the experiment. The result of acute toxicity test phase I and II for both Ethanol and Acetone extracts showed no mortality at 10mg/kg, 100mg/kg and 1000mg/kg; 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. There were no physical signs of distress during the 24 hour period. There was significant weight gain ($p<0.05$) across all the groups. Ethanol extracts group I, II and III recorded a weight gain of 23% while acetone extracts group I, II, and III recorded a weight gain of 11% (Table 2)

Groups	Treatment	Treatment Day 0	Treatment Day 5	Percentage weight gain
Normal saline group	No treatment	20.80±2.18	23.20±2.52	10.50%
Ethanol Extract I	1000mg/kg	17.00±0.63	23.40±1.29	27.35%
Ethanol Extract II	500mg/kg	22.00±1.10	24.40±1.03	9.81%
Ethanol Extract III	250mg/kg	18.80±2.22	19.20±2.50	2.08%
Acetone Extract I	1000mg/kg	17.00±0.45	19.20±0.66	11.46%
Acetone Extract II	500mg/kg	19.20±0.73	21.60±0.93	11.11%
Acetone Extract III	250mg/kg	17.00±0.63	18.20±0.73	6.59%
Experimental control	Chloroquine 5mg/kg	19.20±0.92	18.80±1.59	1.32%
Negative control	Infected, No treatment	18.80±2.20	19.20±2.50	2.08%

Table 2: Effect of Extract on Weight of Experimental Animal
Values are Mean ± SEM

Antiplasmodial activity

Table 3 shows the anti-plasmodial activity of varying oral doses of Ethanol and Acetone extracts compared to Chloroquine and negative control during an early *Plasmodium berghei* infection.The results show a general antiplasmodial activity of the extracts at all concentrations, this is evident from the negative control group with parasitaemia of 23.32%.The result shows significant differences in chemosupression ($p<0.05$) between chloroquine and ethanol extract at 250mg/kg,500mg/kg and 1000mg/kg. However Ethanol at 250mg/kg (93.4%) and Acetone at 250mg/kg (93.81%) showed the highest Chemosupression which was comparable to that of Chloroquine (97.34%). The result shows that the anti plasmodial activity of Ethanol and acetone extracts of Mistletoe are dose dependent.

Table 3: Antiplasmodial Activity of The Extract

Treatment	Dosage (mg/kg)	Parasitaemia (%)	% Chemosuppression
Negative control	Infected,No treatment	23.32±13.05	0
Ethanol Extract I	1000mg/kg	3.05±4.55	86.88
Ethanol Extract II	500mg/kg	7.24±13.08	68.95
Ethanol Extract III	250mg/kg	1.53±0.95	93.4
Acetone Extract I	1000mg/kg	2.58±3.58	88.90
Acetone Extract II	500mg/kg	6.27±10.24	73.11
Acetone Extract III	250mg/kg	1.44±0.39	93.81
Experimental control	Chloroquine 5mg/kg	0.62±0.52	97.34
Normal control	No infection, No treatment	-	-

Values are Mean ± SEM, n=5.

Table 3 shows the average survival time and the total number of dead animals in each group. The average survival time for the animals in the negative control group was 6.8 days; 5 animals died as compared to the Positive control group (28 days); no dead animals. Acetone extract at 250mg/kg had the most number of dead animals(5) and a mean survival time of 17 days, on the other hand, ethanol extract at 1000mg /kg recorded the least number of dead animals (1) and had a mean survival time of 25days. The result shows that the mean survival time of the animals in the experiment did not depend on the doses of the extracts given orally, since the acetone extract at 250mg/kg had the highest chemosupression of 93.81%and most number of dead animals (5).

Ethanol extract at 500mg/kg recorded the highest survival of 27±0.49 days which is similar to that of chloroquine 28±0.36 days.

Treatment group	Dosage (mg/kg)	Mean survival time	Total number of dead animals
Negative control	Infected, No treatment	6.8±1.64	5
Acetone I	1000mg/kg	20±1.57	2
Acetone II	500mg/kg	23±0.34	2
Acetone III	250mg/Kg	17±0.47	5
Ethanol I	1000mg/Kg	27±0.49	1
Ethanol II	500mg/Kg	25±0.36	3
Ethanol III	250mg/kg	22±0.79	3
Chloroquine	5mg/ml	28±0.36	0

DISCUSSION

This work sought to establish a basis for the use of *Tapinanthus. dodoneifolius* (Parkia biglobosa mistletoe) in the treatment of malaria, and to evaluate the potential of its constituents as a possible antimalarial compound and establish a basis for its use in treatment of malaria. This work compared the antiplasmodial activity of a polar (Ethanol) and mid to non-polar (Acetone) extracts of *Tapinanthus. dodoneifolius* with the standard drug, Chloroquine, which has been used for curative treatment of malaria.

The chemosuppression showed by chloroquine in this study was in agreement with other studies on medicinal plants used for such as *Artemisia annua* [18], *Annona senegalensis* [3] and malaria *Adhathoda schimperiana* [22] which showed 90.48, 96.20 and 97.80 %, respectively.

The results from table 3 above indicated that the leaf extracts of *Tapinanthus dodoneifolius* possessed blood schizoncidal activity and therefore anti- malarial activity. This is evident as seen in its ability to suppress *Plasmodium berghei.*

The percentage suppression of parasitaemia of the extract treated groups changed significantly from those in the negative control group showing that the extract has antimalarial activity. Thereby, supporting the folk use of the plant as antimalarial herb; A compound is considered as active when percent suppression in parasitaemia is 30 % or more [15, 2] which supports the findings of the current study.

The extracts prolonged the mean survival time of the study mice indicating that the extracts suppressed *Plasmodium berghei* and reduced the overall pathologic effect of the parasites on the study mice. Accordingly, a plant material that can prolong the survival time of infected experimental animals compared to the negative control group is considered as an active agent against malaria parasite [19].

CONCLUSION

African mistletoe extracts possess good antiplasmodial activity against the parasite tested comparable to that of Chloroquine. The antiplasmodial activities of the extracts were dose dependent although acetone extract recorded a slightly higher antiplasmidial activity than ethanol extract.

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