**Journal of Medical and Biological Science Research**
**Vol. 3 (4), pp. 32-36, September, 2017**
**ISSN: 2449-1810**
**Full Length Research Paper**
**http://pearlresearchjournals.org/journals/jmbsr/index.html**

# Repository Effects of Methanolic Leaf Extract of *Albizia Chevalieri* on *Plasmodium Berghei* using Albino Wistar Rats

**Hajara Sani Labaran[1]*, Samaila A. B.[1], A. F Umar[1], Rabiu Sahal[1], Morumda Daji[1], A. kwaji[2], Paul Mela Leonard[3] and Muktar Adamu Difa[3]**

Accepted 12 September 2017

[1]Department of Biological Sciences, Abubakar Tafawa Balewa University Bauchi, Bauchi State Nigeria.
[2]Department of Chemistry, Gombe State University.
[3]Department of Human Physiology, Gombe State University.

## ABSTRACT

**Malaria is a serious hazard to humanity and the major cause of mortality and morbidity in the malaria endemic countries, and the emergence of resistance to currently used antimalarial drugs makes it imperative to search for newer, more effective therapeutic agents of which *Albizia species* have been reported to have a potential antiplasmodial effect. The purpose of this investigation was to determine the repository effects of methanolic leaf extract of *Albizia chevalieri* on *Plasmodium berghei* using albino wistar rats. The crude methanol extract of *A. chevalieri* leaves was investigated for its antimalarial activity against *P.berghei* (NK65) during established infections. The level of parasitemia, mean survival time was used to determine the antimalarial activity of the extract. The Phytochemical screening of the crude extract were evaluated to elucidate the possibilities of its antimalarial effects. The leaf extract demonstrated significant (P<0.01) dose dependent activity against the parasite and also had repository activity. Leaf extract also prolonged the survival time of the infected rats. It was also revealed that $LD_{50}$ of the plant extract is greater than or equals 3000 mg/kg. The results showed that the leaf extract of the plant has potential antiplasmodial activity, which can be exploited in malaria therapy. Accordingly with the essence of further studies, this plant could serve as a potential source of new and novel antimalarial drug for the control of malaria.**

**Key words: *Albizia chevalieri,* Parasitaemia, *Plasmodium berghei*, Repository and Suppression.**

*Corresponding author. E-mail: slhajara@gmail.com.

## INTRODUCTION

In the absence of effective malaria vaccines, effective chemotherapy remains the mainstay of malaria control. The potentially lethal malaria parasites have shown themselves capable of developing resistance to nearly all used anti-malarial drugs, and resistance strains have rapid extension. For obvious reasons malaria will continue to cause morbidity and mortality on a large scale in tropical and sub-tropical countries, the alarming rise at which the parasite (particularly *Plasmodium falciparum*) have developed resistance to currently used anti-malarial drugs makes it imperative to search for newer, more effective therapeutic agents (WHO, 2000). The loss of effectiveness of chemotherapy constitutes the greatest threat to the control of malaria. Therefore, to overcome malaria, new knowledge, products and tools are urgently needed, especially new drugs (Owolabi et al., 2007; Rasonaivo et al., 1994). The anti-malarial potential compounds derived from plants is proven by example such as quinine, obtained from cinchona species and artemisinins obtained from artemesia Species. Traditional methods of malaria treatment could be promising source of new anti-malarial compounds.

In Africa, more than 80% of people use traditional medicines and most families have recourse to this medicine based on plants extracted for the curative treatment of malaria (Zhang et al., 2000). *Albizia* is a large genus of trees, of the pea family (*fabaceae*), native to warm regions of the old world. The plant *Albizia chevalieri* is a tree that grows up to 12 m high or a shrub under harsher conditions of dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella shaped canopy, bark pale-grayish, twigs pubescent with white lenticles, leaves with 8 to 12 pairs of pinnate and 20 to 40 pairs of leaflets each was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal. The common name of *A.chevalieriis jaree-hi/je*, Hausa name is kasari, is a tree of the dry deciduous forest. Found in well watered places, sandy terraces, not gregarious, nor common (Abdel-Kader et al., 2001).

## MATERIALS AND METHODS

### Collection of Plant Material

Fresh leaves of *A. Chevalieri* were collected from jejin Jigawa around Kumo road, Akko Local Government Area, Gombe State, Nigeria and were duly authenticated by a Botanist using a taxonomic key in the herbarium laboratory in the Department of Biological Sciences, Gombe State University, Gombe, Nigeria.

### Extraction of Plant Material

The leaves of *A. chevalieri* were air-dried at room temperature (25 to 30ºC) under shade for three weeks and were pulverized into coarse particles using pestle and mortar. The coarse particles were further grinded into powder using electric blender. Three hundred grams of the powdered leaves was soaked with absolute methanol (3.5L, Merck, Germany). The extraction process was facilitated in an orbital shaker at 120 rpm for 72 h. The crude extract was filtered twice through cotton wool and then through what man no. 1 filters paper. The filtrate was concentrated at 40ºC using a rotary evaporator and to complete dryness in an aerated oven. The percentage yield was 38.20%. The dry extract was stored in a refrigerator at 4ºC until used (Samuelson, 2007).

### Experimental Animals

White albino Wister rats of both sexes weighing between 100 grams and 200 grams free from infections were obtained from Nigerian veterinary research institute Vom, Jos and were used for the study due to the ease of handling them and obtaining blood. The rats were bred and kept in the animal house, Department of Pharmacology and Clinical Pharmacy, Gombe State University. The animals were housed in cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had free access to water. Treatment of the animals was in accordance with the principles of Laboratory Animal care.

### Malaria Parasites

The Plasmodium specie that was used in this work was *P. berghei* (NK65) which is mostly employed in rodent. The malaria parasite *P. berghei* (NK65) chloroquine sensitive strain was used to assess the antimalarial activity of *A. chavaleiri* leaf extract. The parasite was obtained from National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, the donor rats were kept at the Department of Pharmacology and Clinical Pharmacy, Gombe State University, Gombe. The Parasites were maintained by continuous re-infestation in rats.

### Parasite Inoculation

Donor rat blood infected with the *P. berghei* was used for inoculum preparation. This was done by determining percentage parasitemia and the erythrocytes count of the donor rat, and diluting them with normal saline in proportions indicated by both determinations (Akuodor et al., 2012). The percentage parasitemia was determined by using the formula:

$$\%\ Parastemia = \frac{Number\ of\ infected\ RBCs}{Total\ number\ of\ RBCs} \times 100$$

Percentage suppression was determined by the formula

$$\%\ Suppression = \frac{\%\ parastemia\ of\ negative\ control - parastemia\ of\ treated\ group}{\%\ parastemia\ of\ negative\ control}$$

Each rat was inoculated intraperitoneally with infected blood suspension (0.2 ml) containing $1x10^7$ *P. berghei* parasitized red blood cells.

### Repository Test

This was assessed by using the method described by Awe and Makinde (1997); Adzic and Salawu (2009). Thirty Wister albino rats of both sexes weighing (100 to 200 g) were randomly divided into six groups of 5 rats per cage for methanol leaf extract of *A. chevalieri.* The rats were intraperitoneally administered with various doses of the extract (100,200,400 and 800 mg/kg) which were reconstituted with distilled water and the doses determined by weighing the rats. 80 mg/kg artemether was administered to the reference group and 0.2 ml normal saline to control group, once in a day for three consecutive days. On the fourth day, the rats were passaged

**Table 1.** Repository effect of methanolic leaf of extract of *A. chevaleiri* in rats.

| Drug | Dose (mg/kg) | Non parasiteems density (D7) | % Suppression |
|---|---|---|---|
| Normal saline | 0.2ml | 25.6+0.5 | - |
| *A. chevaleri* | 100 | 6.2+0.3 | 50* |
| | 200 | 4.4+0.1 | 75* |
| | 400 | 2.1+0.0 | 85* |
| | 800 | 1.4+0 | 90* |
| Arthemeter | 80 | 1.0+0.1 | 97* |

D7=Day seven, *significantly different from control at P<0.05 (n=5).

**Table 2.** Effects of methanolic leaf extract of *A.chevalieri* on the mean survival times of *P. berghei* infected rats.

| Groups | No. of animals | Treatment | Dosage (mg/kg) | Death | Mean survival times |
|---|---|---|---|---|---|
| Group 1 | 5 | Artemether | 80.00 | 0 | 1.00 |
| Group 2 | 5 | Normal Saline | 0.20 | 2 | 0.40 |
| Group 3 | 5 | *A.chevalieri* | 100.00 | 1 | 0.80 |
| Group 4 | 5 | *A.chevalieri* | 200.00 | 0 | 0.80 |
| Group 5 | 5 | *A.chevalieri* | 400.00 | 0 | 1.00 |
| Group 6 | 5 | *A.chevalieri* | 800.00 | 0 | 1.00 |

intraperitoneally with standard inoculum of *P. berghi* containing 1x10$^7$ infected erythrocytes. Seventy two hours later (D7), thin films were made from the tail blood of each rat. The films were fixed with methanol, stained with 10% Giemsa and Parasitemia level was assessed microscopically by counting the parasitized erythrocytes on at least 1000 erythrocytes in 10 different fields. Acute toxicity test was performed to ensure that the dose does not have toxic effect on the rats. The mean survival time was calculated immediately at the first day by using the formula

$$Mean\ survival\ Time = \frac{Number\ of\ dead\ rats\ in\ a\ group}{Total\ number\ of\ rats\ in\ each\ goups}$$

### Statistical Analysis

Result obtained were expressed as mean + S.E.M. The significance of difference between the control and treated groups were determined using one-way analysis of variance (ANOVA) (P<0.05) were considered to be statistically significant (Betty and Stern, 2003).

## RESULTS

The methanolic leaf extract of A. *chevalieri* exhibited significant (P<0.05) dose dependent reduction in parasitemia density of 50, 75, 85 and 90% at 100, 200, 400, and 800 mg/kg, respectively, whereas artemether treated group caused 97% reduction in parasitemia density in the test (Table 1).

### Effect of Methanolic Leaf Extract of *A. chevalieri* on the Mean Survival Time

In this study, rats treated with 200, 400 and 800 mg/kg body weight and that treated with the standard drug at a dose of 80 mg/kg body weight had lived longer than the negative control group (Table 2).

### Acute Toxicity Study of Methanolic Leaf Extract of *A. Chevalieri* in Albino Wistar Rats

Acute toxicity evaluation of the leaf extract of Albizia *chevalieri* at various dosage from 250mg/kg to 30000mg/kg body weight showed to be safe as no death was recorded nor visible signs of toxicity nor mortality after 24, 48 and 72 hours in the initial and second phase of acute toxicity. The plant leaf extract might be considered very safe since there were no effect during the acute toxicity study, as shown in table 3 and 4.

## DISCUSSION

In the established infection, the methanolic leaf extract at various doses showed significant dose dependent schizonticidal activity. The observed antimalarial effect of the leaf extract is consistent with the use of the plant as herbal medication against the disease and indication of its potential as a chemotherapeutic antimalaria agent. The extract might be considered very safe since there were no observed unto wards effects during the toxicity tests. This is in agreement with the findings of Rasoanaivo et al. (1994) who reported it to be greater than 3000 mg/kg in rats. The plant extract has a noteworthy antimalarial activity as the mean survival time values at does used were twice or more than that of control group. The result of the current study revealed that the leaf extract of *A. chevalieri* prolong the survival time of an infected rat in the four day suppressive test. Plant materials that can prolong the survival time of infected experimental animals compared to the negative control are considered active

**Table 3**. Initial phase of acute toxicity test.

| | Weight | | Death |
|---|---|---|---|
| GRP 1 | $R_1$ 195 | 0.48=0.5 | None death |
| | $R_2$ 185 | 0.46=0.5 | |
| GRP2 | $R_1$ 168 | 0.84 | |
| | $R_2$ 158 | 0.755 | |
| GRP 3 | $R_1$ 168 | 1.68 | |
| | $R_2$ 164 | 1.64 | |

**Table 4.** Second phase acute toxicity test.

| Group 1 | Ratio | Weight (kg) | Dose of administration | Effect |
|---|---|---|---|---|
| 1 | $R_1$ | 0.260 | 1500 mg/kg | |
| | $R_2$ | 0.260 | | |
| 2 | $R_1$ | 0.282 | 2000 mg/kg | |
| | $R_2$ | 0.270 | | |
| 3 | $R_1$ | 0.298 | 2500 mg/kg | |
| | $R_2$ | 0.300 | | |
| 4 | $R_1$ | 0.330 | 3000 mg/kg | |
| | $R_2$ | 0.315 | | |

agents against malaria (Oliveira et al., 2009). The result obtained from this study showed significant decrease in parastemia of *P. berghei* after treatment with the leaf extract of *A. chevalieri* and this significant decrease in parastemia observed was also dose dependant. When a standard antimalarial drug is used in rats infected with *P. berghei*, it suppresses the parastemia to a non-detectable level (Kiseko et al., 2000).

The percentage suppression of parastemia of the extract treated groups changed significantly from those of the negative control showing that the extract has an antimalarial activity supporting the folk use of the plant as antimalarial herb, a compound is considered as active when percentage suppression in parastemia is 30% or more (Krettli et al., 2001). This also showed that the effect of the extract is comparable with the standard drug. The genus *Albizia* has been reported to contain Alkaloids, glycosides, sapponins, (Varshney et al.,1976), phenolic acids, p-hydroxybenzoic acid, Vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, salicylic acid, quercetin, eugenol and kaempferol (Thomas et al., 1998) which could be responsible for their antimalarial activity. Accordingly, with the essence of further studies this plant could serve as the potential source of new and novel antimalarial drugs for the treatment and prevention of malaria. The leaf extract of *A. chevalieri* demonstrates significant ($P<0.01$) activity in all the models of antimalarial evaluations. The results of this study provided a basis for further studies on the plants. These include the isolations and characterization of the bioactive principles with the ultimate objective of finding novel antimalarial compounds which can be used in the fight against drug resistant malaria. The findings lend pharmacological support to the folkloric use of the plant in the treatment of malaria.

## ACKNOWLEDGEMENT

All Grace is due to Allah, the lord of the world who sustains my efforts throughout the season of my studies and may His salutation be on to our noble Prophet Muhammad (S.A.W). I would like to express my profound gratitude to my able supervisors; Dr. A. B. Samaila and Dr. A.F Umar who despite their tight schedules, monitor the progress of this work and provided useful suggestions, encouragement, constructive criticisms, guides and some relevant literature for the successful completion of this research work, may God reward them interminably. I'd also like to acknowledge my husband and my parents who inspire me throughout my life and who without their support; all would have been in vain. May Allah reward them with Aljannatil-Firdausi. I will also want to say thanks to Gombe State University for allowing me to carry out this research in their laboratory, and their staff especially their technicians for providing me with all the necessary assistance needed throughout my research work, hence I gratefully acknowledged you all.

## REFERENCES

Abdel-Kader M, Hoch J, John M, Evans R, Janes S, Stephen WD, Janes, M, Kingston GI (2001). Two biologically active saponins from Albizia subdimidiata from the Suriname rainforest. J.Nat. prod. 64:536-539.

Adzic B, Salawu OA (2009). Screening Diospyros mespiliformis extract for antimalaria potency. Int. J. Biochem. Sci. 3(2): 271-276.

Ajose F (2007). Some Nigeran plants of dermatologic importance. Int. J. Dermatol. 46 supplementary (1):48-55.

Akuodor GC, Essien AD, Essiet GA, Essien DO, Akpan JL, Udoh FV (2013). Evaluation of antipyretic potential of pseudocedrela kotschyi schweint. Harms (meliaceae). Eur. J. Med. Plants 3(1):105-113.

Abdel-Kader M, Hoch J, John M, Evans R, Janes S, Stephen WD, Janes

M, Kingston GI (2001). Two biologically active saponins from Albizia subdimidiata from the Suriname rainforest. J.Nat. Prod. 64:536-539.
Kiseko K, Matsuoka H, Furuhata SI, Fujisaki RI, Kawakami T, Mogi S,Shibuya T (2000). Anti-malarial activity of leaf-extract of Hydrangea macrophylla, a common Japanese plant. Acta Medica Okayama, 54(5): 227–232.
Krettli A (2009). Antimalarial drug discovery: screening of Brazilian medicinal plants and purified compounds. Expert opinion in Drug Discovery, 4: 95-108.
Oliveira AB, Dolabela MF, Braga FOC, Jacone RIRP, Varotti FP, Pora MM (2009). Plant-derived antimalarial agents: new leads and efficient phytomedicines. Anais da Academia Brasileira de ciencias,81(4): 715-740.
Owolabi MO, Ogah OS, Ogunniyi A (2007). Episodic vertigo resulting from vascular risk factors, cervical spondylosis and head rotation: Two case reports. Neuropsych. Dis.Treat.3(5):675-678.
Rasoanaivo P, Ratismamanga-Uverg S, Milijaona R, Rafatro H, Rakoto-Ratsimamanga A, Gallefi C, Nicoletti M (1994). In vitro and invo chloroquine-potentiating action of strychnos myrtoids alkaloids against chloroquine resistant strains of Plasmodium malaria. Planta Medica.60(1):13-16.
Thomas AM, Van Der Wel AM, Thomas AW, Janse CJ, Water AP (1998). Transfection systems for animal models of malaria. Parasitol Today, 14: 248-249.
Varshiney I, Pal R and Vyas P (1976). Studies on lebbekanin E a new saponin from Albizia lebbeck Benth. J. India Chemical Soc., 53: 859-860.
World Health Organization (WHO) (2000). Promoting the role of traditional medicine in health systems: A strategy for the African Region 2001-2010.Harare.
Zheng L, Zheng J, Zhao Y, Wang B, Lijun W, Liang H (2006). Three anti-tumor saponins from Albizia Julibrissin. Bio.org. Med.Chem. Lett. 16:2765-2768.