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EVALUATION OF ANTIMALARIAL ACTIVITIES OF THE ACETONE LEAF EXTRACT OF *Ochna rhizomatosa* (Ochnaceae) IN *Plasmodium berghei* INFECTED MICE

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ABSTRACT
Ochna rhizomatosa (van Tiegh.) Keay [Ochnaceae], a plant used in the treatment of malaria in Northern Cameroun was evaluated for antimalarial activity. The antimalarial activity of acetone extract of Ochna rhizomatosa leaf obtained through cold maceration at room temperature were evaluated in albino mice infected with chloroquine-sensitive Plasmodium berghei in order to justify its activity or performance as antimalarial remedy. Activities evaluated were suppressive effect and curative effect. The acute toxicity test gave an LD_{50} value of 86 mg/kg. The in vivo antimalarial effect of the extract (86.0, 43.0 and 21.5 mg/kg body weight) against P. berghei showed significant ($p < 0.05$) dose-dependent activity for both suppressive and curative tests. Result of blood schizonticidal activity of the acetone extract in suppressive test gave percent parasitaemia inhibition activity of 40.0%. While the curative test gave a dose dependent percent parasitaemia inhibition activity of 46.44%, highest at the highest administered dose. The schizonticidal performance were comparable to that of chloroquine which had percentage suppression of parasitaemia of 68.8%. The percentage mean survival time of the P. berghei infected mice treated with extract doses were comparable to that of the mice treated with the standard drugs (chloroquine). The result showed that the herbal extract possesses significant antimalarial potency which was comparable to that of standard antimalarial drugs used.
Keywords: Antimalarial activity, Plasmodium berghei, Parasitaemia inhibition, Ochna rhizomatosa, Schizonticidal activity.

INTRODUCTION

Malaria remains one of the most infectious and deadly diseases worldwide, caused by the *Plasmodium* parasite. The World Health Organization (WHO) reported about 247 million cases from 84 malaria endemic countries and 619,000 estimated malaria deaths in 2021 (WHO, 2022). Most malaria deaths were reported in the WHO African region, with almost 76% of the total deaths recorded in children under 5 years old (WHO, 2022). In endemic areas, the most vulnerable target population for malaria disease includes children and pregnant women [WHO, 2022, Woldearegai *et al.,* 2019*,* Maghendji-Nzondo *et al.*, 2016). However, the malaria burden persists among residents of perennial transmission zones, where asymptomatic carriers are reportedly vast parasite reservoirs and are often adults because of acquired immunity within the exposure time and age (Bouyou-Akotet *et al.,* 2010).

Following the official implementation of artemisinin-based combination therapy (ACT) under the WHO's recommendation to reduce the risk of drug resistance in 2003, the first-line treatment for non-severe *P. falciparum* malaria has been artesunate + amodiaquine (AS + AQ) or artemether–lumefantrine (AL), and sulfadoxine–pyrimethamine (SP) has been the intermittent preventive treatment (IPT) for pregnant women (Maghendji-Nzondo *et al.*, 2016, Kun *et al.,* 1999, Ramharter *et al., 2007*). Various studies have evidenced that there is drug failure of SP which is linked to point mutations, including $N_{51}I$, $C_{59}R$, $S_{108}N$, and $I_{164}L$ in *dihydrofolate reductase* (*dhfr*) and $S_{436}A/F$, $A_{437}G$, $K_{540}E$, $A_{581}G$, and $A_{613}T/S$ in *dihydropteroate synthase* (*dhps*) (Mawili-Mboumba *et al.,* 2013, Gesase *et al.,* 2009). Since the introduction of these artemisinin-based drug combinations, polymorphisms in *dhfr* and *dhps*, which are linked to their resistance, have been continuously spreading across the nation in different regions (Kayode *et al.,* 2021, Desai *et a.,*/ 2016, Bouyou-Akotet *et al.,* 2010). An ethnomedicinal survey was conducted between February 2017 and January 2019 in agreement with the executive committee of the Federation of National Associations of Traditional Medicine Actors of Benin (FANAMETRAB), Republic of Benin. The work was authorized by the Research Ethics Committee of the Interregional University of Industrial Engineering, Biotechnology, and Applied Sciences under the number B001331455. (Codo *et al*., 2022). Plants species used are with a technicality that varies depending on the type of condition to be treated (wounds, fractures, or sprains) with the same preparations. The most cited among the recorded species were *Ochna rhizomatosa* and *Ochna schweinfurthiana* (Nonvignon *et al*., 2022). The roots and stem bark of *Ochna rhizomatosa* have been identified as a plant used for the massage of the ribs in the form of a decoction in Ghana and Cameroon for the treatment of wounds, which strengthens its ethnopharmacological use in the Republic of Benin in the treatment of bone fractures, wounds, and sprains [Wada *et al*., 2012]. However, the stem bark has reported to be used in Cameroon for the treatment of malaria, jaundice, wound and intestinal helminthiasis (Betti, 2011). This research work tried to validate the traditional use of the plant *Ochna rhizomatosa* also as an antimalarial.

MATERIALS AND METHODS:

Collection, identification and preparation of plant materials.

The leaf part of *Ochna rhizomatosa* (van Tiegh.) Keay (Ochnaceae) was collected from Karaukarau village, Zaria in the month of June, 2012. The plant was identified and authenticated at the Herbarium Section of Biological Science Department, Ahmadu Bello University, Zaria, Nigeria, as *Ochna rhizomatosa,* after comparism with reference specimen with a voucher number of 2760. The fresh plant leaves were air-dried and made into powder using pestle and mortar.

Extraction

The powdered plant leaf (500g) of *Ochna rhizomatosa w*as extracted with acetone to exhaustion using cold maceration at room temperature. The solvent was removed *in-vacuo* to afford 51.15g of the acetone extract, equivalent to 10.23%, a dark greenish – brown gummy mass known as acetone extract (Dieter and Victor, 2013. The extract gave dark green colour with alcoholic ferric chloride, orange colour with Zn –hydrochloric acid test. This clearly indicates that this extract contains flavones nucleus. Preliminary phytochemical examination of the extract of the leaves of *Ochna rhizomatosa* indicates the presence of flavonoids, glycosides, tannins, saponins steroids and carbohydrates. However, alkaloids are found to be absent in this specie.

Experimental Animals

The animals were obtained from National Institute for Tryponosomiasis Research, Kaduna and bred in the animal house of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria to attain a weight between 15 – 25g.

Infected animals

Swiss albino mice infected with *Plasmodium berghei* parasites were obtained from Nigerian Institute of Medical Research, Lagos, Nigeria. The animals were fed with standard mouse cubes and tap water. The parasite stock was maintained by continuous re-infection in the bred mice.

Standard Drug

Chloroquine used as positive control was obtained from May and Baker.

Instrument

Light microscope was used to count the parasite in the blood smear.

Acute toxicity study (LD_{50}) of the Acetone Extract.

LD_{50} determination was carried out using the method of Chinedu (2013). Thirteen mice were used for this study in the first phase, three doses of the extract (10, 100 and 1000 mg/kg) were administered intraperitoneally (i.p) to three groups each containing three mice each. In the second phase, four doses of the extract (600, 370, 225 and 140 mg/kg) were administered through the same route to four groups containing one mouse each.

The median lethal dose (LD_{50}) value was calculated as the thirty percent of the square root of the product of the highest non-lethal dose and the lowest lethal dose of the extract.

The experimental design involved two distinct experimental protocols: -

Evaluation of blood schizonticidal activity in suppressive test:

This was done using a method similar to that described by Leslie (2007). A total of 30 mice were used for this study on blood schizonticidal activities. Each mouse was subsequently given standard intra - peritoneal inocula of $1.02x10^5$ *P. berghei* parasites (Chloroquine sensitivity) with the aid of a 1ml disposable syringe. This was done to all the mice. The animals were then divided into five groups of six mice each. The treatments were all given intraperitonially. Group 1 to 3 animals were given 21.5, 43 and 86 mg/kg per day of the extract respectively. Group 4 animals were given 5 mg/kg per day of Chloroquine and group 5 animals were given 0.2ml of normal saline. All the extracts, drug and distilled water were given for three days. Group 4 served as the positive control group while group 5 served as the negative control group. All the extracts, drugs and distilled water were given for 3 days. On the fourth day, thick blood smears were made from the blood samples obtained from the tails (caudal veins) of the animals. The smears were stained with Giemsa stain and examined under the light microscope for the levels of parasitaemia. The average percentage suppression of parasitaemia was calculated in comparism to negative control using this formular;

Chemosuppression of parasite growth = $100 - \left(\frac{\text{Mean parasitemia treated}}{Mean\ parasitemia\ control}\right)\ X\ 100$

Evaluation of blood Schizonticidal activity in curative tests:

This was determined using a method similar to that described by Getnet (2022). The mice were divided into 5 groups (a - e) consisting of 6 mice each. All the animals were inoculated intraperitoneally with $1.02x10^5$ *Plasmodium berghei* parasites with the aid of a 1ml disposable syringe. Treatment was commenced on the fourth day (72hrs later) using 21.5, 43 and 86 mg/kg per day of extract for group a, b and c respectively. Group 'd' animals were given 5mg/kg per day of Chloroquine intraperitoneally and group 'e' animals were given 0.2ml of normal saline. All the extracts drug and distilled water were given for three days. Group 'e' served as the negative control group. The treatment using the extract, drug and normal saline were given for three days. After 24 hours of completion of the treatments, thick blood smears were made and the level of parasitaemia determined as described above.

Statistical analysis:

Results obtained were presented as mean ± standard error of mean. Statistical analysis was done using student's t-test. A 'p' < 0.05 were considered significant. The mean survival period for each group within a 28days period was determined and noted.

RESULTS

The acute toxicity studies result is as shown in Table 1. The mice were observed to showed general CNS depression, restlessness and subsequently death. The LD_{50} value was found to be 86mg/kg. A total of 25% of the animals survived after 28days of inoculation. The blood schizoticidal activity is as shown in Table 2 and shows the various doses of O. rhizomatosa acetone leaf extract, for the suppressive test, using chloroquine as standard drug. For this suppressive test, it shows that the average percentage chemosuppression of *O. rhizomatosa* at the highest dose administered was found to be 40%. The result of *Plasmodium berghei* on curative test is as shown in Table 3 and it shows that the average percentage chemosuppression of *O. rhizomatosa* at the highest dose administered was found to be 46.66%.

Table 1: Acute toxicity study (LD_{50}) of the extract

Group	Dose of extract (mg/kg)	No of mice	No of Dead
1	140	6	0
2	225	6	0
3	370	6	0
4	600	6	6

LD_{50} **=** √highest LD x √lowest non LD x 30%

= √600 x √370 x 30 /100

= 86mg/kg

Table 2: Effect of suppressive test on *Plasmodium beighei* parasite.

Treatment	Dose (mg/kg)	Mean ± SEM of parasitaemia	%Parasitaemia Inhibition	Significant Difference (t- test)
N/S	0.2*	3.75 ± 0.09		
Extract	21.5	2.25 ± 0.84	40	P < 0.05
Extract	43	2.25 ± 0.09	40	P < 0.5
Extract	86	2.25 ± 0.41	40	P < 0.05
Chloroquine	5	1.17 ± 0.29	68.8	P < 0.05

Key: * = ml

Table 3: Effect of curative test on *Plasmodium beighei* parasites.

Treatment	Dose (mg/kg)	Mean ± SEM of parasitaemia	% Parasitaemia Inhibition	Significant difference (t-test)
N.S	0.2*	3.75 ± 0.55	_	_
Extract	21.5	2.20 ± 0.55	41.33	P < 0.05
Extract	43	2.16 ± 0.58	42.40	P < 0.05
Extract	86	2.00 ± 1.15	46.66	P < 0.05
Chloroquine	5	1.17 ± 0.29	68.8	P < 0.05

Key: * = ml

DISCUSSION:

This suppressive test which had 40% activity was found to be significant when compared with the positive standard drug (chemosuppresion is 68.8%) using students t- test. The `p' value less than 0.05 was significant. This curative test which had 46.66% activity was also found to be significant when compared with the positive standard drug (chemosuppresion is 68.8%) using students t- test, where a `p' value less than 0.05 is significant.

CONCLUSION:

The acetone leaf extract of *O. rhizomatosa,* showed that the extract has antimalarial property of which the activity was found to be significant when compared with the standard drug (Chloroquine) using student's t- test.

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