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IN VITRO ANTIPLASMODIAL ACTIVITY OF METHANOLIC ROOT EXTRACT OF BOERHAAVIA DIFFUSA ON CHLOROQUINE SENSITIVE PLASMODIUM FALCIPARUM

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ABSTRACT

This study evaluated the antimalarial effect of *B. diffusa* root extract on chloroquine sensitive *P. falciparum*. This was with a view to providing additional scientific information on its use as an antimalarial agent. The crude methanolic root extract of the plant was tested for its *in vitro* anti-plasmodial activity against *Plasmodium falciparum* (chloroquine-sensitive strain). In this study, 100 µl culture media were dispensed into a 96-well microtitre plate, leaving the 'A' series. Then, 100 µl of the test extract was pipetted into well 'A' and double fold serial dilutions were made down to well 'H'. The last series 12 wells served as control. Twenty five (25) µl of infected red cells was separately added into all the wells in the test plates and mixed by swirling with the tips to ensure homogeneity and incubated at 37^0C in a candle jar and assessed for parasite growth after 27 h of incubation. The results of the *in vitro* assay showed that the methanolic extract of *B. diffusa* possessed weak antimalarial activity against *P falciparum* with IC_{50} of 55.35 µg/ml when compared with the standard antimalarial drug chloroquine whose IC_{50} was 0.5209 µg/ml. The antiplasmodial activity of the roots of the plant supports local claims on its efficacy in the treatment of malaria infection.

KEYWORDS: Boerhaavia diffusa, Plasmodium falciparum, Malaria, Antiplasmodial activity.

INTRODUCTION

Malaria is a parasitic disease and one of the most important diseases in the tropical and subtropical regions and it is known to be the greatest cause of hospitalization and death among the children of age 6 months to 5 years.[1] Each year, there are approximately 515 million cases of malaria, killing between one and three million people, the majority of whom are young children in Sub-Saharan Africa.[2] Compared to a century earlier, the area of malaria risk has reduced from 53% to 27% of the earth's land surface and the number of countries exposed to some level of malaria risk has fallen from 140 to 106. The alarming rate at which *P. falciparum* has developed resistance to chloroquine and other synthetic antimalarial drugs makes it necessary to search for more effective antimalarial compounds.[3] In Africa and other countries where malaria is endemic, traditional medicinal plants are frequently used to treat or cure malaria.[4] It is a fact that conventional antimalarials such as chloroquine, quinine and artemisinin derivatives originated from plants.[5] It is therefore important to investigate the antimalarial activity of medicinal plants in order to determine their potentials as sources of new antimalarial agents.[6] *Boerhaavia diffusa* is a creeping herbaceous plant traditionally known as *B. diffusa* in india and also named in Brazil as Erva tostao. It is about 1 m long with spreading branches, belonging to the Nyctaginaceae family. In the Ayurvedic herbal medicine, the root of the plant is used against gonorrhea, internal inflammation, oedema, anaemia, dyspepsia, liver abdominal pain, gall bladder and kidney disorders and even cancer.[7] The plant is orally taken to purify blood and relieve muscular pain[8] In Nigeria, it is widely used for the treatment of epilepsy.[9] Pharmacological evaluation of extracts of B. *diffusa* has shown that it possessed anti-inflammatory[10], diuretic[11], laxative[12], anti-urethritis[13], anticonvulsant[14], anti-nematodal[15], anti-bacterial[16], anti-hepatotoxic.[17] Though various pharmacological activities of *B. diffusa* have been investigated, yet its antimalarial activity against *Plasmodium falciparum* has not yet been elucidated, hence, this study.

MATERIAL AND METHODS

Plant Material and Preparation of B. *diffusa* Extract

Sample of *B. diffusa* was obtained from a dunghill behind Olatunde Memorial Nursery and Primary School Bolorunduro, Ilesa in Ilesa East Local Government, Osun State, Nigeria on 15th August 2012 by 3 pm and

transported to the Herbarium Unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria for identification and given IFE Herbarium voucher number, 16900. A specimen of the leaves was kept in the department's herbarium under the same voucher number. The roots of the plant were dried in the oven at a temperature under 60^0 C and powdered in the grinding machine while 500 g of the powdered plant was macerated with 2.2 litres of 100 % methanol with intermittent shaking twice in a day for 72 hours and then filtered. The filtrate was concentrated to dryness in vacuo with a rotatory evaporator. The residue was collected and stored in the refrigerator (4^0C) for onward use.

Drugs and Laboratory Materials

Standard chloroquine (Sulphate salt – Batch No. 442), obtained from May and Baker Nigeria Plc., PMB 21049, 3/5 Sapara Street, Industrial Estate, Ikeja, Lagos was employed as standard reference for the antimalarial screening in this study. The materials utilized include weighing balance (Mettler Toledo), grater (machine), oven (Gallenkamp), rotatory evaporator (Shnelheber), refrigerator, 1ml syringes and needles, disposable 0.45 µM millipore and filter unit receiver, RPMI 1640, powdered medium, stored at 4° C, sodium bicarbonate ($NaHCO_3$, stored at room temperature), HEPES (stored at room temperature), gentamicin (stored at -20° C) and a candle jar dessicator.

Experimental Animals

The animals used for acute toxicity test were adult male and female mice (18 – 20 g) obtained from the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Nigeria.

Acute Toxicity

The acute toxicity (p.o. LD_{50}) of the methanolic root extract of *B. diffusa* was estimated in 13 white albino mice using standard method of Lorke.[18] This test was carried out in two phases; In phase 1 study, three groups of 3 mice each were used. The mice in the three groups were orally administered with the doses of 10, 100 and 1000 mg/kg body weight of the test extract respectively and monitored for 24 hours, observing for mortality. In phase 2 study, three groups of 1 mouse in each group were used. The mice in the three groups were orally administered with the doses of 1600, 2900 and 5000 mg/kg body weight of the extract respectively and similarly monitored for 24 hours, observing for mortality. The phase 2 test was carried out based on the result obtained in phase 1 study.

Parasite collection

Sample of *P. falciparum* infected blood (3 ml) was collected into EDTA bottles. The blood was centrifuged at a speed of 3000 rpm for 12 minutes and the plasma was removed. The red blood cell deposit (1 ml) was diluted with 4 ml of incomplete RPMI media to make a solution of 5 ml.

Preparation of the culture medium and working solutions

All glasswares were previously washed thoroughly, well packed with aluminum foil and then sterilized by heating in an autoclave at 120^o C for 30 minutes. The pipettes were washed, dried and sterilized by heating in the oven at a temperature of 170^0 C for 2 hours. RPMI (Rose Packwell Memorial Institute reagent), 2M HEPES (Hydroxy-ethylpiperazine N-12-ethane-sulfonic acid) and sodium bicarbonate ($NaHCO_3$) were added to sterile distilled water under the Lamina flow hood. Gentamycin (40 mg/ml) was also added to the medium, and the mixture was shaken. The pH of the medium was checked at 7.6. The mixture was later filtered using 0.45 mm millipore filter. This makes the incomplete culture medium. Complete medium was prepared by adding ten percent human blood group O^+ serum to the incomplete medium in the ratio 1:10.

Extract (0.5 mg) was dissolved in 2 ml of 95% methanol and diluted in 3ml of distilled water to give a stock solution of 100 mg/ml. From this stock solution, a 2 fold serial dilutions was made with RPMI-1640 medium to give a final concentration of extract (4.57, 13.72, 41.15, 123.46, 370.37, 1111.11, 3333.33 and 10000 µg/ml.).

Chloroquine (40 mg/ml) was diluted with sterile distilled water to make up to 40 mls making a stock solution of 1 mg/ml. From the stock concentration of chloroquine, a 2 fold serial dilutions was made with RPMI-1640 medium to give a final concentration of chloroquine (0.05, 0.14, 0.41, 1.23, 3.71, 11.11, 33.33 and 100 µg/ml.).

***In vitro* cultivation of P. falciparum isolates and sensitivity test**

Culture medium (100 µl) was dispensed into all the wells in a 96-well microtitre plate leaving only the 'A' series. Then, 100µl of the working solutions were pipetted into the well 'A' series. Serial dilutions were then prepared by pipetting 50 µl of the solutions from the 'A' series wells down the rows up to the 'H' series wells, leaving series 12 from wells 'A' down the rows to 'H' only with the culture medium, serving as control. The extra 50 µl of test substances dilution series was discarded.

Infected red cells (25 µl) was separately added into all the wells in the test plates using the multi-channel micropipette and mixed by swirling with the tips to ensure homogeneity. The starting parasitaemia was between 2.5% to 7.2%. Negative controls were performed without the addition of the test samples.

Titre plates were incubated in CO_2 condition at 37 ºC in candle jar for 24-30 hours.[19] After incubation, contents of the wells were harvested and the red cells transferred to a clean microscopic slide to form a series of thick films. The films were stained for 10 minutes in 10 % Giemsa solution of pH 7.2 and the slides were viewed under light microscope (X 100 magnification) with immersion oil to assess the parasites for growth. All

experiments were performed in triplicates and the results were expressed as percentage of growth inhibition. Crude extracts with IC_{50} values > 50 µg/ml were considered to be inactive.[20]

Statistical Analysis

The Microsoft Excel 2007 was used to calculate mean parasite growth and percentage parasite inhibition. The student t-test was used to analyze the data and values of $P \leq 0.05$ were considered significant.

RESULTS

Acute Toxicity

For the acute toxicity investigation carried out, all the animals were still alive at the end of the experiment and the LD_{50} value was greater than 5000 mg/kg body weight and is of no practical interest. Hence, the extract can be non-toxic.[18]

In vitro* antiplasmodial effect of *B. Diffusa* methanolic root extract on *P. falciparum

The effect of *B. diffusa* extract (4.57, 13.72, 41.15, 123.46, 370.37, 1111.11, 3333.33 and 10000 µg/ml.) in *in vitro* assay showed a decrease in the percentage parasitaemia with increasing drug concentration (Figure 1). The basic measurement of antimalarial activity used in this study was the reduction in number of parasitized cells in the test cultures compared to control at 24 - 30 hours of incubation. With regard to concentrations administered, a dose-dependent antimalarial activity was clearly shown for the crude extract. Chloroquine (0.05, 0.14, 0.41, 1.23, 3.71, 11.11, 33.33 and 100 µg/ml.) only also exhibited remarkable antiplasmodial activities (Figure 2). The median inhibitory concentration IC_{50} values for the extract and chloroquine were 55.35 and 0.521µg/mL respectively (Figures 1 and 2) which shows the weak antimalarial activity displayed by the plant on *P. falciparum* when compared to the standard antimalarial drug Chloroquine.

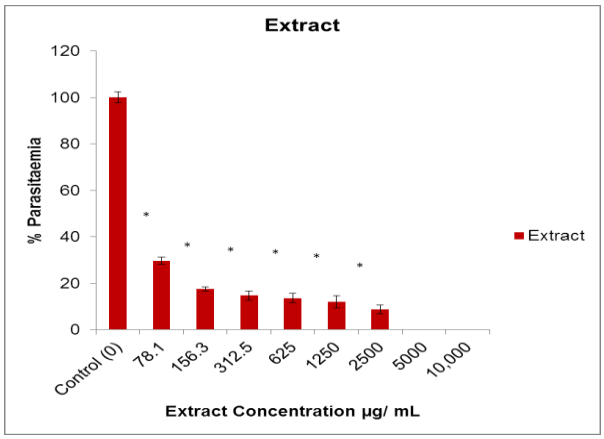

Fig. 1: Effects of different concentrations of methanolic root extract of *B. diffusa* on *in vitro* growth of *P. falciparum*.

Values are expressed as mean ±SEM. From the experiments performed in triplicates.

* indicates $P < 0.05$. IC_{50} extract was 55.35 µg/ml.

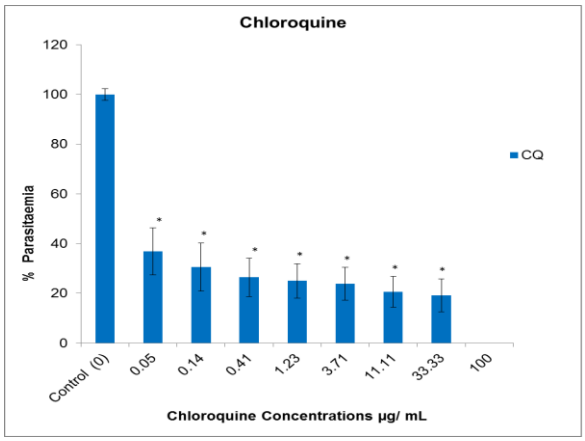

Fig. 2: Effects of different concentrations of chloroquine on *in vitro* growth of *P. falciparum*.

Vaues are expressed as mean ±SEM. From the experiments performed in triplicates. * indicates $P < 0.05$. IC_{50} extract was 0.521 µg/ml.

DISCUSSION

This study investigated the acute toxicity profile of methanolic root extract of *Boerhaavia diffusa*, its antiplasmodial activity on chloroquine sensitive *P. falciparum*. According to the method of Lorke[18], the oral median lethal dose (LD_{50}) of the crude extract was estimated to be ≥ 5000 mg/kg body weight since there were no mortality at all dose levels used. The absence of death following the oral administration of *B. diffusa* root extract at 5000 mg/kg body weight observed in mice suggests that the extract is practically non-toxic.[21] This is also buttressed by[22] that any chemical that exhibited an LD_{50} more than 5000 mg/kg is practically non-toxic. The toxicological profile of this plant is supported by that of the other specie *B. elegans* which showed no toxicity.[23]

In the *in vitro* assay, the IC_{50} of the crude extract of *B. diffusa* was 55.35 µg/mL, suggesting a weak antiplasmodial activity relative to chloroquine phosphate. According to[24], the extract is active when $IC_{50} < 5$ µg/ml, moderately active when 5 µg/ml < IC_{50} < 50 µg/ml and weak when $IC_{50} > 50$ µg/ml. This value is however similar to those obtained for other crude extracts with established antimalarial activity.[25,26] The decrease in parasitaemia with increasing concentration of the extract (Fig. 1) also reflects an inhibitory activity on parasite replication thus supporting the antimalarial activity of the extract.

The antiplasmodial activity of *B. diffusa* observed in this study may be attributed to the presence of some bioactive compounds in its root as reported in a study that *B. diffusa* roots contain some phytochemicals which include alkaloids, glycosides, saponins, flavonoids, polysaccharides, steroids and tannins.[27]

Considering the inhibitory effect of *B. diffusa* root extract on the growth of *P. falciparum* as reported in the result of this study, it is therefore concluded that the

antiplasmodial activity of the plant on *P. falciparum* supports local claims on its efficacy in the treatment of malaria infection. Further study is on-going to isolate, identify and characterize the bioactive compound from the plant.

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