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(RESEARCH ARTICLE)

Anti-*Plasmodium berghei* activity of Ethanol root bark extract of *Hippocratea africana*

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Abstract

Drug resistance is the major challenge that has undermined the viability of the synthetic anti-malaria drugs. The active ingredients inherent in plants are distributed to the various parts which can only be explored based on available information on them. Thus, the aim of this study was to evaluate the anti-plasmodium berghei activity of ethanol root bark extract of *Hippocratea Africana*. Freshly harvested root bark of *H. Africana* was dried at room temperature and afterwards ground into fine powder. 500 g of the powdered plant sample was extracted. Twenty five adult mice were divided into five (5) mice each. **Group I** was the normal control (NC), **Groups II-V** were infected with *Plasmodium berghei*. However, while group II was not treated with extract, **Groups III and IV** were treated with 200 and 400 mg/kg of ERBEHA respectively and **Group V** was administered 250 mg/kg of chloroquine. Animals were sacrificed at the termination of administration of extract and blood sample collected was analysed using standard procedures. *P. berghei* infection significantly ($P<0.05$) increased percentage parasitemia, reduced total protein and packed cell volume, oral administration significantly ($P<0.05$) reversed the observation. In conclusion, it can be deduced from the outcome of this work that the root bark of *H. Africana* wields the ability to eliminate *Plasmodium berghei*.

Keywords: *Hippocratea Africana; Plasmodium berghei*; Chloroquine; Drug resistance; Root

1. Introduction

Malaria, a deleterious disease known to be caused by plasmodium parasite (WHO, 2015) has been identified as the main cause of death predominantly in developing countries notably in the endemic Sub-Sahara Africa. An estimated 445 deaths resulting from 216 million cases of malaria were reported in 2016 globally (2017), while in Nigeria, it is implicated in about 110 million clinical cases and an estimated 300,000 deaths yearly which translates to 60% of all outpatients attendance and 30% of all hospital admissions (FMH, 2015).

Drug resistance is the major challenge that has undermined the viability of the conventionally used antimalarial drugs and has reawakened the abandoned age long practice of relying on plants for the treatment of malaria (Hostettmann *et al*., 2000). The therapeutic components of a plant are often distributed to different plant parts such as the root, flower, stem bark and the leaf in different concentrations which largely determines how effectively it can be explored by the people to whom it is available depending on their knowledge of the degree of efficacy obtainable from each part. More so, the fact that the prevailing climatic condition of a place determines what plant grows at such an area orchestrates an unending search for therapeutic plants as a particular therapeutic plant in a particular area may be lacking in another geographical area.

Hippocratea africana is an inhabitant of the green forests, a perennial climber which has hairs known as glabrous. It is reproduced from seed (Dalziel, 1956). The plant is widely distributed in tropical Africa and the roots are used

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traditionally in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea (Okokon et al. 2006). Phytochemical analysis on parts of the said plant shows that it is an embodiment of alkaloids, cardiac glycosides, tannins, anthraquinononones and flavonoids (Nden et al. 2014).

2. Material and methods

2.1. Experimental Animals

Twenty five (25) adult mice were procured from the animal house of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Uwana Afikpo. The animals were treated and handled in the most humane manner. They were housed in transparent plastic cages and were fed guinea grower feed for two week to acclimatize them before the experiment.

2.2. Malaria parasite

Four mice passaged with the parasite *Plasmodium berghei* used for this study was procured from the Deparment of Microbiology, University of Nigeria Nsukka.

2.3. Collection of plant sample

The root bark of *Hippocratea africana* was obtained from Uturu, Abia State and was subsequently identified at the Department of Forestry, Michael Okpara University of Agriculture Umudike, Southeastern Nigeria.

2.4. Extraction

Bark of freshly harvested roots of *H. africana* were thoroughly washed with tap water to remove sand and afterwards peeled off with the aid of a cutlass before being dried at room temperature. 465 g of the plant material was ground and sieved to obtain fine powder which was subsequently steeped in 1000 mL methanol, stirred 24 hourly for 72 h. The extracts obtained was filtered, first through muslin cloth then through filter paper and subsequently concentrated to a gummy material under reduced pressure at 50°C with the aid of a rotary vacuum evaporator.

2.5. Acute Oral Toxicity Study

The guidelines of the Organization for Economic Cooperation and Development (OECD 425, 2008) were employed to determine the acute toxicity of extract using limit test dose of 2 g/kg. A total of five healthy non-pregnant female Swiss albino mice which had been restricted from food for 4h were dosed and afterwards weighed to determine the dose. The first animal was administered a limit dose of 2000 mg/kg and no death was observed within 24 hours of dosing. Another 4 female mice were dosed and observed for signs of toxicity such as diarrhea, weight loss and absence of tremor, lethargy, and paralysis periodically for the first four hours during the 24-hour period and later were followed for 14 days for any lethality (OECD, 2020).

2.6. Parasite passaging

The method described by Peter and Anatoli (1998) was employed to innocultate *Plasmodium berghei* (NK 65) into the mice by intraperitoneal route (Anatoli, 1998). Red blood cells infected with *Plasmodium berghei* was collected from the tail vein of the infected mice and was subsequently diluted in 5 mL of phosphate buffered saline (PBS), so that 1 mL of parasitized blood contains 5 x 10^9 RBC m^{-1} infected erythrocytes, each 0.2 mL of the blood that was subsequently injected contained 1 x 10^6 *Plasmodium berghei* parasitized red cells (Huang et al., 2015). Administration of extract began three (3) days after inoculation.

2.7. Animal grouping

- **Group I:** (Normal control) apparently healthy mice fed with rat chow and water only.
- **Group II:** *Plasmodium berghei* infected mice without treatment
- **Group III:** *Plasmodium berghei* infected mice treated with 200 mg/kg bw of the aqueous extract of *H. africana.*
- **Group IV:** *Plasmodium berghei* infected mice treated with 400 mg/kg bw of the aqueous extract of *H. africana.*
- **Group V:** *Plasmodium berghei* infected mice administered with 250 mg/kg chloroquine.

2.8. Malaria microscopy

Parasite density was determined by the method of Warhurst and Williams (1996) on each group on day 0, 3, 5 and 7 by preparing thick blood film. This was done by collecting blood from the distal end of the tail, with sterile scissors. Three

drops of the blood samples were placed on a clean slide, a thin blood film was made. These films were air dried, fixed in methanol for 30 seconds and stained with Gimsa stain for about 4 minutes (Cheesebrough, 2014). The slides were thoroughly rinsed under running tap and left to dry. The prepared slides were viewed under X 100 objective oil immersion light microscope. Parasitemia level was determined using the formula below:

$$\text{Parasitemia} = \frac{\text{Total number of parasitized red blood cell x 100}}{\text{Total number of red blood cell}}$$

2.9. Packed Cell Volume Determination

With the aid of the heparinized microhematocrit capillary tubes, blood was obtained from the tail of each mouse. $3/4^{th}$ of the height of the capillary tubes were filled with blood and afterwards sealed at one end using sealing clay. The tubes were subsequently loaded onto a microhematocrit centrifuge, with the sealed end outward and centrifuged for 5 minutes at 11 000 rpm, after which they were read using a standard microhematocrit reader. PCV was measured after infection had been established (Dikasso *et al*. 2006).

2.10. Biochemical analysis

2.10.1. Serum total protein

Evaluation of serum total protein concentration was performed by biuret method. In this approach, alkaline copper reacts with the peptide bonds of proteins to form a characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation and potassium iodide prevents the auto reduction of copper. The color intensity is directly proportional to protein concentration. The absorbance was read at 546 nm. The concentration of serum total protein was expressed as g/dL.

2.10.2. Serum albumin

The serum albumin concentration was measured using the bromocresol green method described by Dumas et al. (1971). The intensity of the green color is proportional to the concentration of albumin present in the sample and was expressed as g/dL.

2.10.3. Serum globulin

The serum globulin level was determined by subtracting the albumin value from the corresponding value of total protein. The concentration of serum globulin was expressed as g/dL.

2.11. Statistical analysis

Data generated from the study was analyzed using statistics software IBM SPSS Statistics 21. Data were expressed as mean ± standard deviation (SD). The results were considered significant at $P<0.05$. Mean values were compared using one way analysis of variance (ANOVA).

3. Results

Table 1 Percentage Parasitemia and Packed Cell Volume of *P. berghei* infected Mice treated with Ethanol Root Bark Extract of *Hippocratea africana*

% Parasitemia					
Groups	**Treatments**	**Day 3**	**Day 5**	**Day 7**	**PCV**
Group I	NC	0.00±0.00a	0.00±0.00a	0.00±0.00a	45.0 ± 11.70b
Group II	INF-UNT	76.66±5.77d	83.33±5.77e	96.66±5.77c	19.1 ± 5.32a
Group III	200 mg/kg EEHA	56.66±5.77c	36.66±5.77d	13.33±5.77b	44.0 ± 11.00b
Group IV	400 mg/kg EEHA	40.00±10.00bc	23.33±5.77c	13.33±5.77b	44.0 ± 11.00b
Group V	25 mg/kg CQ	36.66±5.77b	10.00±0.00b	0.00±0.00a	47 ± 10.34c

Results are expressed as mean ± standard deviation of three determinations. Values with different superscript in a column are significantly different at ($P \leq 0.05$).

Table 2 Serum Protein Level of P. berghei infected Mice administered with Ethanol extract of Root bark Extract of Hippocratea Africana

Grouping	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)
Group I (Normal control)	81.9 ± 21.52^{c}	29.2 ± 8.03^{b}	52.1 ±13.26^{cd}
Group II (Negative control)	48.2 ± 14.34^{a}	21.7 ± 5.71^{a}	27.0 ± 8.32^{a}
Group III (200 mg/kg extract)	72.9 ±19.48^{b}	30.7 ± 8.15^{b}	42.2 ±14.00^{b}
Group IV (400 mg/kg extract)	74.6 ± 17.96^{b}	34.1 ± 7.68^{c}	41.2 ± 10.55^{b}
Group V (Standard drugs)	78.2 ± 16.60^{bc}	30.2 ± 7.72^{b}	48.1 ± 10.89^{c}

Results are expressed as mean ± standard deviation of three determinations. Values with different superscript in a column are significantly different at (P≤0.05).

4. Discussion

Effective utilization of a plant for the treatment of a disease solely depends on the practitioners' or patients' knowledge about the localization of the active ingredients of interest within the plant and oftentimes, a viable plant may be underutilized if the right part is not used in the treatment of a disease. Table 1 shows the percentage parasitemia and packed cell volume (PCV) obtained on the red blood cell of *P. berghei* orally administered with ethanol root bark extract of *H. africana* showing that the percentage parasitemia of infected mice progressively increased over a seven day period. However, oral administration of ethanol root bark extract of *H. africana* significantly ($P<0.05$) reduced parasitemia in a dose dependent manner. This could be attributed to the phytochemicals notably the alkaloids and tannins reportedly present in the plant. This finding is consistent with the outcome of a research made by Okokon et al (2006). Packed cell volume is one of the indicators of malaria infection. It was evaluated to determine the effectiveness of the said extract in preventing hemolysis resulting from rising parasitemia levels. The packed cell volume (PCV) reported on the *P. berghei* infected group was significantly ($P<0.05$) low evident by the fact that malaria parasite caused lysis of red blood cells (Kotepul et al 2014). However, following oral administration of the said extract, the PCV increased significantly ($P<0.05$) which could be due the antiplasmodial activity of the extract on the parasitized RBCs and the causative organism thereby sustaining the availability of new RBCs produced in the bone marrow (Kaur et al. 2009).

Research efforts have established the relationship between malaria induced stress and the level of protein. Table 2 shows the serum protein level of *P. berghei* infected mice administered with ethanol root bark extract of *H. africana* showing that *P. berghei* infection significantly ($P<0.05$) reduced total protein (albumin and globulin). This is consistent with the finding of Adebisi et al (1998) who reported significant decrease in plasma total proteins in malaria patient compared to normal individuals. However, this was reversed following oral administration of ethanol root bark of *H. africana* in a dose dependent manner. This is could be attributed to the elimination of the parasite from blood circulation.

5. Conclusion

The outcome from this study, shows that the ethanol root bark extract of *Hippocratea africana* have the ability to clear from *Plasmodium berghei* from the blood of an infected organism and thus can be considered a candidate plant for the development of a novel anti-malaria therapy.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that no conflict of interest exists.

Statement of ethical approval

Ethical approval was obtained from the University Committee on the care and handling of laboratory animals.

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