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Effects of Medicinal Plants on Acetylcholinesterase Activity in the Blood of Mice Infected with *Plasmodium berghei*

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Abstract

Plasmodium species and acetylcholinesterase (AChE) is implicated in cerebral malaria, which is the cause of 12% of psychiatry disorder and a leading cause of death in sub-Saharan Africa. In this study we examined the AChE activity in the blood of mice infected with *plasmodium berghei* (*P. berghei*) and treated with leaf extracts of *T. occidentalis*, *V. doniana, V. amygdalina, O. basilicum* and Artesunate respectively. The parasitemia level and PCV evaluated; and the AChE activity in each mouse were determined using acetylthiocholine as substrate. There was significant ($p< 0.05$) increase in AChE activity in the extracts groups (0.0491-0.1573, 0.0431-0.2061, 0.0327-0.2031, 0.0513-0.0992 × 10^{-3} µmol ACTC min^{-1}mg $protein^{-1}$ respectively) and the standard drug (0.044-0.147 × 10^{-3} µmol ACTC min^{-1}mg $protein^{-1}$) of mice infected and treated for 7 days compared with the Normal and Disease control groups (0.3898-0.3898 and 0.156-0.0888 × 10^{-3} µmol ACTC min^{-1}mg $protein^{-1}$ respectively). The parasitemia level in the disease control group increases with days, which significantly ($p< 0.05$) reduces in groups that received plant extracts and Artesunate treatment. The PCV was stabilized in the treatment groups after an initial reduction. This finding reveals that the blood AChE activity decreases during infection of *P. berghei*, which was reversed upon administration of plant extracts and Artesunate. The increase in enzyme activity inversely correlates with reduction in parasite load. The findings have major implications for understanding how the plant extracts interact to enhance resistance to *P. berghei* proliferation in the body system.

Keywords

Plasmodium berghei, Acetylcholinesterase, Parasitemia, Plant Extracts, Artesunate

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1. Introduction

Malaria is an infectious disease that arises from the presence of parasitic protozoa of the genus plasmodium within the RBC [1]. In 2016, an estimated 216 million cases of malaria occurred worldwide, leading to 445,000 deaths [2]. The disease remains the most prevalent malaria parasite in the tropical and sub-tropical regions of the world and is transmitted by the female anopheles mosquito [1, 2]. It is one of the oldest and greatest health challenges affecting 40% of the world's population, making it a major global health problem with high mortality and morbidity [3, 4]. The disease causes a remarkable economic burden in many developing nations, and treatment of these parasitic diseases is by use of drugs as there are no approved vaccines in the market [5, 6].

Malaria parasite is one of the most thoroughly studied intracellular parasites, yet relatively little is known of the metabolic and regulatory interaction between the parasite and the host cell [7]. Infection of mice with *Plasmodium berghei*

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and Plasmodium yoelii is one of the first models used to test efficacy of antimalarial agents [8].

Cholinesterases are large family of enzymatic proteins widely distributed in both neuronal and non-neuronal tissues, with the principal role of rapid hydrolysis of acetylcholine, thus terminating cholinergic nerve impulses [9, 10]. Recent findings support the enzyme's role in mediating the processing and deposition of Aβ peptide by colocalizing with Aβ peptide deposits in the brain of Alzheimer's disease patients and promoting Aβ fibrillogenesis through the formation of stable AChE-Aβ complexes [11]. The formation of these complexes promotes Aβ aggregation as an early event in the neurodegenerative cascade of Alzheimer's disease [12], and results in cognitive impairment in doubly transgenic mice expressing human amyloid precursor protein (APP) and human AChE [13, 14]. While the physiological role of AChE in neural transmission has been intensively studied and its functions in health and disease condition recognized, it is still the focus of pharmaceutical research targets in the treatments of myasthenia gravis, alzheimer and many other diseases [15, 16].

Synthetic drugs such as artesunate, has been proven and validated for use in the management of malaria infection. However, the emergence and spread of artemisinin-resistant Plasmodium falciparum is a major concern for malaria control [17] thus, antimalarial research continues due to the resistance of malaria parasite to mainstay antimalarial drugs. According to WHO, about three quarters of the world population depends on traditional remedies for the health care of its people. In Africa, for example up to 80% depends on traditional herbs [18]. The new health agenda in Nigeria and in Africa focuses on the institutionalization of traditional medicine in parallel with orthodox medicine into natural health care scheme, in order to advance the health agenda, since effective health care may not be achieved in Africa by orthodox medicine alone [19]. One of the widely used plants for traditional medicine is *Telfairia occidentalis, Vitex doniana, Vernonia amygdalina and Ocimum basilicum*.

2. Materials and Methods

2.1. Chemicals

Acetylthiocholine (ATC), 5, 5-dithiobis [2-nitrobenzoic acid] (DTNB), were obtained from Sigma (St. Louis, MO). All analytical-reagent grades were purchased from Merck (Darmstadt, Germany).

2.2. Animals

Swiss Albino mice weighing 16 – 30g of both sexes were obtained from the Animal House of the College of Health Science, Benue State University, Makurdi, Nigeria, and the guide for the care and use of Laboratory Animal, 1996 of the Institute of Laboratory Animal Research Commission on life Science, was duly followed. The animals were kept in standard cage and acclimatized for 14 days under ambient environmental conditions. They had free access to chick grower's mash and water *ad libitum*.

2.3. Malaria Parasite

The malaria parasite (*Plasmodium berghei*) was obtained from the Department of Parasitology, Ahmadu Bello University, Zaria, Nigeria, were a stabilate was maintained. Parasitized erythrocyte was obtained from donor mice by cardiac plexus puncture and was diluted with tri-sodium citrate prior to intraperitoneal inoculation with 0.3 mL blood suspension containing 10^5 - 10^6 parasitized erythrocytes on day 0.

2.4. Experimental Design

A total of 35 mice were used. The mice were randomly divided into 7 groups of 5 mice each, and labeled 1, 2, 3, 4, 5, 6 and 7. Group 2 was inoculated with *plasmodium berghei* parasitized erythrocytes and not treated. Group 3 was inoculated with the parasitized erythrocytes for 5 days, followed by treatment with 100mg of Artesunate. Group 4, 5, 6 and 7, were inoculated with parasitized erythrocytes for 5 days, followed by treatment with 50mg of *T. occidentalis, V. doniana, V. amygdalina and O. basilicum* respectively. Group 1, was the normal control mice which was not inoculated and not treated, but only given vehicle of administration.

2.5. Determination of Packed Cell Volume (PCV)

Blood was drawn from the tail of the mice in the different groups using heparinized capillary tubes. Duplicate determinations of the hematocrit packed cell volume were done to determine the relative volume of blood occupied by erythrocyte using the equation below:

$$\text{PCV} = \frac{\text{Volume of erythrocytes in a volume of blood}}{\text{Total blood volume}}$$

2.6. Parasitemia Determination

A smear film of thin blood were made from blood collected from the tail of each mouse during and after the experiment in accordance with the method described by Fidock *et al*. [20]. The smear made on microscopic slides were fixed in methanol and stained with Giemsa, and the numbers of parasitized erythrocytes in each 10 – 60 field were counted thrice and the average was taken to represent the parasitemia level of each mouse.

2.7. Acetylcholinesterase Activity Determination in the Blood

AChE enzymatic assay was carried out in the blood cells, by introducing small modifications to the method described by Srikumar *et al.* [21].

Exactly 3.0ml of blood cells (in 0.1M phosphate buffer, pH 7.0) was added into cuvette containing 25µL of DTNB and was incubated for 2 minutes. The content was mixed thoroughly by bubbling air and the absorbance measured at 436 nm using spectrophotometer. When absorbance reached a stable value, it was recorded as the basal reading. 20µl of substrate (acetylthiocholine) was added and change in absorbance was recorded for a period of 10 mins at interval of 2 mins. Change in the absorbance per minutes was determined.

The activity of the enzyme was calculated using the formula:

$$\text{Specific activity} = \frac{\Delta A412 \times \text{VolT} \times 1000}{1.36 \times 104 \times \text{light path} \times \text{Vols} \times [\text{Protein}]}$$

ΔA_{436} = change in absorbance per minutes

Vol_T = total assay volume (DTNB + sample + ACT)

1.36×10^4 = extinction coefficient of DTNB (M^{-1} cm^{-1})

Light path = 1cm

Vols = sample volume (ml)

[Protein] = concentration of protein (mg/ml)

2.8. Protein Determination in Blood Homogenate

Protein concentrations of the homogenates were determined by methods described by Bradford, [22]. Using bovine serum albumin as the standard. 1.0ml of the blood homogenate was separately mixed with 3.0ml Bradford reagent and the absorbance measured at 595nm. The absorbance was used to extrapolate the concentration of protein from the standard curve of protein prepared with BSA.

2.9. Data Analysis

Results were presented as mean±SEM. Within and between groups, comparisons were performed by the analysis of variance (using SPSS 20.0 for windows computer software package). Significant differences were compared by Duncan's new Multiple Range Test, with significance difference set at $p<0.05$ [23].

3. Results

The results indicate that, animals in the infected, untreated groups showed increases in parasite load with a corresponding reduction in AChE activity as the infection progressed. The 7-day suppressive treatment carried out with 50mg of the plant extracts and 100mg/kg artesunate presented a marked reduction in parasite load when compared with the infected, untreated controls (Figure 1).

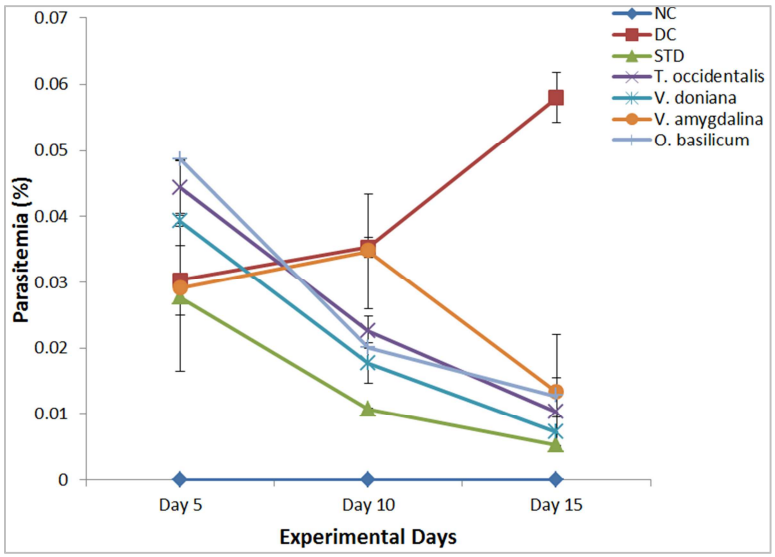

Figure 1. Percentage parasitemia profile of experimental animals.

Packed cell volume (PCV), which is the volume percentage (%) of red blood cells was significantly reduced as shown in Table 1. The infected, untreated experimental controls mice present reduction in packed cell volume when compared with the normal control group, as the days of infection increases. However, administration of plant extracts and artesunate to infected groups ameliorated the drastic reduction in PCV when compared with the initial values.

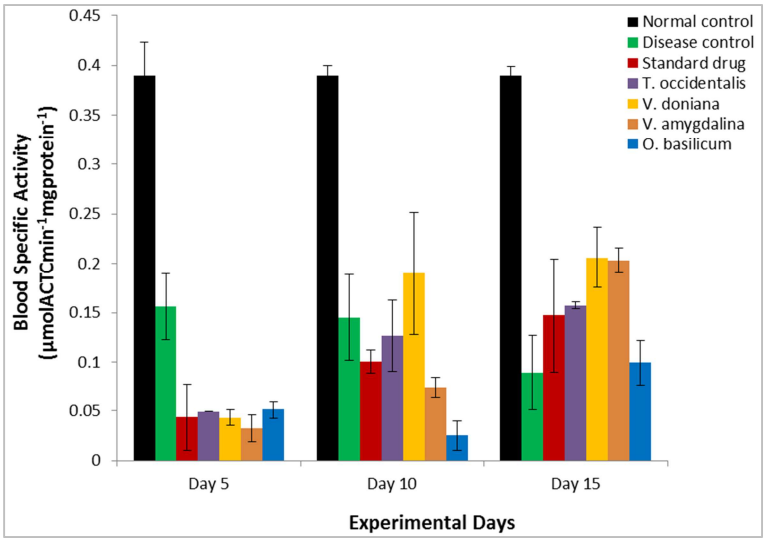

Figure 2. The AChE activity in the blood homogenates.

Table 1. Packed Cell Volume (%) profile of experimental animals.

Groups	Day 5	Day 10	Day 15
Normal control	40.5±6.5^a	40.5±6.5^a	40.5±6.5^a
Disease control	28.5±2.5^a	22±3^b	25.5±5.5^b
Standard drug	39±1^a	47.5±2.5^b	47.5±1.5^b
T. occidentalis	29.5±7.5^a	37±2^b	40±1.0^b
V. doniana	25±1^a	52±3^b	47±2^b
V. amygdalina	36±0^a	31±1.2^b	39±1.2^a
O. basilicum	20±0^a	37.5±2.5^b	37.5±7.5^b

Values are expressed as mean ± SEM of five determinations. Values with different superscripts along the rows are significantly different at $p<0.05$.

Figure 2 presents the AChE activity in the blood of infected and uninfected mice. It was discovered that AChE activity decreases with an increase in progression of parasite load/day. However, the decrease in enzyme activity was significantly ($p<0.05$) halted upon administration of plant extracts and artesunate as compared with the control group, thus, *Vitex doniana* provides the best activity.

4. Discussion

Acetylcholinesterases (AChE) terminate neurotransmission at cholinergic synapses by splitting the neurotransmitter acetylcholine (ACh). The enzyme demonstrates a high degree of variability in distribution, with its notable presence in non-neuronal tissues [24]. Therefore, estimating the AChE activity provides valuable information on cholinergic functions. The mechanism of action of the plant extracts in this experiment may be similar to that of Artesunate, a standard antimalarial drug which induces the destruction of the asexual forms of the plasmodium parasite. Artesunate in this study was observed to decrease the plasmodium organisms in the infected mice.

The findings showed that the PCV decreased after infection with *Plasmodium berghei*. This is an indication of anaemia, due to haemolysis. Anaemia is a consistent finding in blood protozoan parasites infection. Some earlier investigators have observed a progressive fall in PCV values in plasmodium infections [25]. In the present study, the PCV values appear to improve with the plant extracts and artesunate treatment which may be due to the inhibitory effect of the plants/ standard drug on the plasmodium organisms. Substances with antianemic properties are known to stimulate increased production of RBC and improve the values of PCV [26].

In the present study, the significant decrease in the specific activity of AChE in the blood of infected untreated mice with *P. berghei*, suggest possible reduction in the enzyme activity. Thus, an increased in the amount of ACh that persist in the synapses, similar report was corroborated by Noor, [27]. The decrease in the activity of AChE in the blood of infected mice was sustained with the duration of untreated infection, and this could probably explain the elimination of motor dis-coordination and paralysis seen in animals infected with cerebral malaria [28]. However, upon treatment with the plant extracts and Artesunate, there was increased in the enzyme activity which corresponded to decreased parasites load.

The efficacy of plant extracts and several other standard drugs such as Artesunate has been reported, using validated animal models to suppress parasitemia to undetectable levels in mice infected with *P. berghei* [5, 20, 30], which agrees with the present study. Artesunate often produces a rapid clinical and parasitological response to infections which led to immense interest for its use in the management of malaria [30]. In this study, the treatment of *P. berghei* infected mice with 50mg plant extracts and 100mg/kg of artesunate showed

potency in suppressing parasitemia level, and inversely boosting acetylcholinesterase activity in the blood of infected mouse, thus accounting for ameliorating effect.

5. Conclusion

This result can be utilized to predict the action of the plant extracts, as well as a variety of antimalarial drugs. The findings have major implications for understanding how the plant extracts and artesunate interact to enhance resistance to *P. berghei* proliferation in the body system. Our opinion at this time is that the antimalarial potential of the plant extracts and artesunate could in part be linked to the enhancement of AChE activity.

References

[1] Ibrahim HN, Imam IA, Bello AM, Umar U, Muhammad S, Abdullahi SA. The potential of Nigerian medicinal plants as antimalarial agent. *Inter J Sci Tech* 2012; 2 (8): 600-605.

[2] Linares M, Postigo M, Cuadrado D, Ortiz‐Ruiz A, Gil‐Casanova S, *et al*. Collaborative intelligence and gamification for on-line malaria species differentiation. *Malar J* 2019; 18 (21): 1-9.

[3] Wolfgang WL, Elke SB, Evelina A. Comparison of *plasmodium berghei* challenge models for evaluation of pre-erythrocytic malaria vaccines and their effect on perceived vaccine efficacy. *Malaria J* 2010; 9 (145): 1-12.

[4] Habluetzel A, Pinto B, Tapanelli S, Nkouangang J, Saviozzi M, *et al*. Efects of *Azadirachta indica* seed kernel extracts on early erythrocytic schizogony of *Plasmodium berghei* and pro-infammatory response in inbred mice. *Malar J* 2019; 18 (35): 1-9.

[5] Isma'il S, Amlabu E, Andrew JN. Antimalaria effect of the ethanolic stem bark extracts of *Ficus platyphylla* Del. *J Parasitol Res* 2011; Article ID 618209, 5 pages.

[6] Nyamai DW, Tastan OB. Aminoacyl tRNA synthetases as malarial drug targets: a comparative bioinformatics study. *Malar J* 2019; 18 (13): 1-27.

[7] Mark FW, Patricia AW, John WE, Sheppard JR. Membrane-associated phosphoproteins in *plasmodium berghei*-infected Murine erythrocytes. *The J Cell Biol* 1988; 97: 196-201.

[8] Ronan J, Fatima E, Valery C. Georges EG. In-vitro culture of plasmodium berghei-ANKA maintains infectility of mouse erythrocytes inducing cerebral malaria. *Malaria J* 2011; 10 (346): 1-5.

[9] Zatta P, Ibn-Lkhayat-Idrissi M, Zambenedetti P, Kilyen M, Kiss T. *In vivo* and *in vitro* effects of aluminum on the activity of mouse brain acetylcholinesterase. *Brain Res Bul* 2002; 59 (1): 41-45.

[10] Rajasree PH, Ranjit S, Sankar C. Screening for acetylcholinesterase inhibitory activity of methanolic extract of *Cassia fistula* roots. *Inter J Pharm Life Scie* 2012; 3 (9): 1976-1978.

[11] Inestrosa NC, Alvarez A, Perez CA, Moreno RD, Vicente M, Linker C, Casanueva OI, Soto C, Garrido J. Acetylcholinesterase accelerates assembly of amyloid-β-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* 1996; (16): 881-891.

[12] Inestrosa NC, Dinamarca MC. Alvarez A. Amyloid-cholinesterase interactions. Implications for Alzheimer's disease. *FEBS J* 2008; 275: 625-632.

[13] Rees T, Hammond PI, Soreq H, Younkin S, Brimijoin S. Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex. *Neurobiol Aging* 2003; 24: 777-787.

[14] Rees TM, Berson A, Sklan EH, Younkin L, Younkin S, Brimijoin S, Soreq H. Memory deficits correlating with acetylcholinesterase splice shift and amyloid burden in doubly transgenic mice. *Curr Alzheimer Res* 2005; 2: 291-300.

[15] Srikumar BN, Ramkumar K, Raju TR, Shankaranarayana R. Assay of acetylcholinesterase activity in the brain. *Brain Behav* 2004; 142-144.

[16] Shin-Hua L, Josephine WW, Hsuan-Liang L, Jian-Hua Z, Kung-Tien L, Chih-Kuang C, Hsin-Yi L, Wei-Bor T, Yih H. The discovery of potential acetylcholinesterase inhibitors: A combination of pharmacophore modeling, virtual screening, and molecular docking studies. *J Biomed Scie* 2011; 18 (8): 1-13.

[17] Artitaya T, Phunuch M, Wanna C, Kesara N, *et al*. Antimalarial Activity of Piperine. *J Trop Med* 2018: 1-7.

[18] World Health Organisation. Legal status of traditional medicines and complementary alternative medicine: A worldwide review, 2011.

[19] Elujoba AA, Odeleye OM, Ogunyemi CM. Traditional medicine development for medical and dental primary health care delivery system in Africa. *Afri J Trad Alter Med* 2005; 2: 46-61.

[20] Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. *Nat Rev Drug Discov* 2004; 3 (6): 509-520.

[21] Srikumar BN, Ramkumar K, Raju TR, Shankaranarayana R. Assay of acetylcholinesterase activity in the brain. *Brain Behav* 2004: 142-144.

[22] Bradford MM. A rapid sensitive method for quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal Biochem* 1976; (72): 248-254.

[23] Duncan D. Multiple Range and Multiple F Tests. *Biomet* 1955; 11: 1-42.

[24] Tripathi A, Srivastava UC. Acetylcholineaterase, a versatile enzyme of the nervous system. *Annals Neuroscie* 2008; 15: 106-110.

[25] Ajagbonna OP, Esaigun PE, Alayande NO, Akinloye AO. Anti-malarial activity and haematological effect of stem bark water extract of *Nuclea latifolia*. *Bioscie Res Comm* 2002; 14 (5): 481-486.

[26] Brown BA. Hematology principles and procedures. 2nd Edition. Philadelphia, Lea and Febiger, 1976; pp. 200.

[27] Noor NA. Anxiolytic action and safety of Kava: Effect on rat brain acetylcholineaterase activity and some serum biochemical parameters. *Afr J Pharm* 2010; 4: 823-828.

[28] Aikawa M, Iseki M, Barnwell JW, Taylor D, Howard RJ. The pathology of human cerebral malaria. *Ame J Med Hyg* 1990; 43 (2): 30-37.

[29] Kamei K, Matsuoka H, Furuhata SI. Anti-malarial activity of leaf-extract of *Hydrangea macrophylla*, a common Japanese plant. *Acta Medica Okayama* 2000; 54 (5): 227-232.

[30] Dondorp AM, Day NP. The treatment of severe malaria. *Trans R. Soc Trop Med Hyg* 2007; 101 (7): 633-634.