

Short Communication

Effect of Di-Herbal Mixture of *Annona Squamosa* Linn. and *Alstonia Boonei* De Wild On Some Biochemical Parameters of Malaria Infected Albino Mice

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Abstract

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The effect of di-herbal mixture of *Alstonia boonei* and *Annona Squamosa* (two plants traditionally used in the treatment of malaria) on serum aspartate aminotransferase (AST), alanine amino-transferase (ALT) and total protein of malaria infected mice was studied. Mice of both sexes (n = 30), weighing between 24 – 36g were inoculated with Chloroquine sensitive *Plasmodium berghei* infected erythrocytes, each mouse receiving about 1×10^7 P. *berghei* parasites. 72 hours after parasite inoculation, the animals were randomly distributed into five treatment groups, A – E (n = 6 each). Groups A – C were treated with the herbal mixture at respective doses of 400mg/kg, 600mg/kg and 800mg/kg while groups D and E received 5mg/kg chloroquine and 5ml/kg normal saline respectively. Treatments lasted for five days. On the sixth day, blood samples were taken from the animals for serum biochemistry. Animals treated with the herbal preparation showed serum levels of the parameters which were very similar to that of the animals treated with the standard drug (chloroquine). The untreated animals however, showed significant elevations in the values of these parameters. These findings thus support the combination of the two herbs for higher synergistic antimalarial effect.

Keywords: *Alstonia boonei*, *Annona Squamosa*, biochemical parameters, *plasmodium berghei*, mice.

INTRODUCTION

The World Health Organization recently listed Nigeria among high burden countries with limited evidence of decrease in malaria cases (WHO, 1977; Soniran et al., 2012). Malaria is a disease caused in humans by parasites of the plasmodium species through the bite of infected female anopheles mosquito. About 3.3 billion people, half of the World's populations are at risk of malaria. Everyday, this leads to about 250 million malaria cases and nearly one million deaths (Soniran et al 2012). In attempt to tackle the problem of malaria, a lot of effort has been made by man, ranging from the use of standard

orthodox medicines to the use of crude preparations made from plant parts. Among such plants used to treat malaria in Nigerian folk medicine are *Alstonia boonei* and *Annona Squamosa*.

Annona squamosa commonly known as custard apple or sweet sop is a semi-evergreen shrub or small tree reaching 6-8meters (20-26ft) tall. The plant is a native of tropical America and the West-Indies, but its original home is uncertain. It is said to have been introduced to Bahia, Brazil, in 1626. Planted and naturalized in Southern Florida, including Florida Keys and throughout

the tropics in Asia and South Pacific. It has run wild, particularly near old inhabited sites, in several parts of the central and Western India and in the Decan Peninsula.

Annona Squamosa has a wide array of ethno botanical uses. Fruits are normally eaten fresh. The roots are cathartic and purgative. The tree is a good source of firewood. Green fruits, seeds and leaves have effective vermifugal and insecticidal properties. The leaves, shoots, bark and roots have been reported to have medicinal properties. (Kirtika et al 1957). The plant is also traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, bacterial infection, fever and ulcer (Kirtika et al 1957). *Annona squamosa* has been reported to exhibit antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. A number of antimalarial compounds have been isolated from *A. squamosa* which is traditionally used in diseases including infections associated with malarial parasites (Johns et al., 2011).

Alstonia boonei is a widespread genus of evergreen trees and shrubs from the dog-bane family (*Apocynaceae*). It is commonly known as Cheesewood, Pattern wood or Stool wood. In Nigeria, it grows in moist low land forests. Among the medicinal uses of the plant are as antidiuretic, spasmolytic and hypotensive (Oliver, 1986). *Alstonia boonei* has been widely used in recipes to treat malaria. (Idowu et al 2010; Titanji et al 2006).

In our earlier work, we have observed a significant reduction in parasitaemia when a preparation made from combined extracts of *A. boonei* and *A. squamosa* is administered to *Plasmodium berghei* parasite infected mice up to a dose of 800mg/kg body weight. The present study is an evaluation of the effect of the herbal mixture on the serum AST, ALT and Total protein levels of infected mice as further evidence in support of its earlier observed antiplasmodial effect.

MATERIALS AND METHODS

Plant materials

Fresh leaves and root bark samples of *Alstonia boonei* were collected from Amaimo in Ikeduru area of Imo State Nigeria. Fresh leaves of *Annona squamosa* were collected from Nekede in Owerri West area of Imo State Nigeria. All samples were identified by a taxonomist in the department of Biotechnology, Federal University of Technology Owerri.

Preparation of Plant Materials

The fresh root barks of *A. boonei* were cleaned, cut into

pieces and air-dried under shed for two weeks. They were subsequently milled to powder using a mechanical blender.

The fresh leaves of *A. squamosa* were dried under shed for one week. The samples were later milled to powder using a mechanical blender.

Extraction of Plant Materials

250g of each ground sample was weighed out and mixed together to give 500g of mixed herbal powder. This was soaked in 1500ml of 95% methanol for 72 hours, at the end of which filtration was done using filter paper. The filtrate was subsequently concentrated in a rotary evaporator at 45-50°C to yield a residue which was stored in a refrigerator at 4°C

Animal Treatment

Thirty Swiss albino mice of both sexes weighing between 26 – 38g were used for the experiment. They were sourced from the animal holdings of the department of Biochemistry University of Port Harcourt and acclimatized in the laboratory for two weeks before commencement of study. They were fed with standard palette diet and water ad libitum. The United States National Institute of Health "Principles of Laboratory Animals Care (NIH 1978) were adhered to in the study.

Malaria Parasites

Chloroquine sensitive *Plasmodium berghei* parasites were sourced from the department of Biochemistry, Nigerian Institute of Medical Research, Yaba, Lagos Nigeria. Albino mice previously infected with *P. berghei* served as parasite donors.

Inoculation of Parasites

At the end of the acclimatization period, each of the thirty mice was inoculated with parasitized donor erythrocytes containing about 1×10^7 *Plasmodium berghei* parasites. 72 hours after parasite inoculation, the animals were randomly distributed into five groups (A – E) of six mice per group. The animals were treated as follows:

Group A (400 mg/kg herbal mixture), Group B (600mg/kg herbal mixture), Group C (800mg/kg herbal mixture), Group D (5mg/kg Chloroquine phosphate) and Group E (5ml/kg normal saline). These treatments were given once daily for five consecutive days (Riley and Peters 1970). 24 hours after the end of treatment, blood samples were taken from the animals for measurement of parasitaemia and serum biochemistry.

Table 1. Provide table legend

Treatment	AST Activity (i.u/L)	ALT Activity (iu/L)	Total Protein (g/dL)
Normal Saline 5ml/kg bw	84.17±1.47 ^a	75.17±1.54 ^a	5.42±0.42 ^c
400mg/kg b.w.	74.33±2.28 ^b	59.88±4.97 ^b	6.43±0.50 ^{bc}
600mg/kg b.w.	63.90±3.01 ^c	49.50±1.41 ^c	6.98±0.58 ^{abc}
800mg/kg b.w.	39.92±3.43 ^d	32.67±2.45 ^d	7.62±0.87 ^{ab}
Chloroquine (5ml/kg b.w)	39.33±3.14 ^d	31.17±2.23 ^d	8.00±0.85 ^{ab}

Data are Mean ± SEM (n=6)

Means in the same column with different superscripts differ significantly (P< 0.05)

RESULTS AND DISCUSSIONS

Our earlier findings revealed pronounced elevations in parasitaemia levels of malaria parasite infected and untreated mice and converse reductions in parasitaemia levels on treatment of infected mice with the herbal mixture. Histological examinations showed a severe distortion of the cyto-architecture of the liver parenchyma in the malaria infected but untreated animals thus confirming the deleterious effect of malaria on the liver. In the current study, malaria infected (but untreated) animals showed pronounced elevations in serum levels of AST and ALT, but reductions in total protein also indicating negative impact of malaria on the liver. The other groups of animals equally infected with malaria but treated with the herbal mixture however, showed reductions in levels of AST and ALT with converse increase in total protein values similar to animals whose malaria was treated with a standard drug, chloroquine phosphate. These observations thus serve as more evidence in support of the continued use of the herbs in malaria treatment. It is also recommended that both herbs be used together for stronger synergistic antimalarial effect

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