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# Effect of *Alstonia Boonei* and *Annona Senegalensis* Combination on Histopathological Damages Caused by Plasmodium Berghei in the Liver of Infected Albino Mice

**Onwusonye J. C.**
Department of Biochemistry, Federal Polytechnic, Nekede-Owerri Imo State, Nigeria
**Uwakwe A. A.**
Department of Biochemistry, University of Port Harcourt, Rivers State Nigeria
**Iwuanyanwu P.**
Department of Biochemistry, University of Port Harcourt, Rivers State Nigeria

***Abstract***
*The effect of combined extracts of Alstonia boonei and Annona Senegalensis (two plants traditionally used in the treatment of malaria) on histopathological damages caused by plasmodium berghei on the liver of infected mice was studied.*
*Mice of both sexes (n = 30), weighing between 26 – 38g were inoculated with Chloroquine sensitive Plasmodium berghei infected erythrocytes, each mouse receiving about 1 x $10^7$P. berghei parasites. 72 hours after parasite inoculation, the animals were randomly distributed into five treatment groups, A – E (n = 5 each). Group A – C were treated with the herbal preparation 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, the mice were sacrificed and liver samples fixed in 4% formaldehyde for histological study adopting H & E staining procedure. Animals treated with the herbal preparation showed relatively normal liver histological features similar to that of the animals treated with the standard drug (chloroquine). The untreated animals however, showed severe distruction of the hepatocytes with marked necrosis. These findings thus support our earlier observed antimalarial efficacy of the herbal preparation.*

***Keywords****: Alstonia boonei , Annona Senegalensis, histopathological damages, plasmodium berghei, mice.sssssssss*

## 1. 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 Senegalensis.*

*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*, 2008).

*Annona senegalensis* is a plant commonly known as "African Custard apple", "Wild Sour Sop", or "Wild Custard apple". It is a flowering plant in the family *Annonaceae*. The plant is commonly used in Nigeria folk medicine as a remedy for malaria. The roots are also commonly used in treatment of conditions like dizziness, indigestion, chest cold and veneral diseases (Fabricant and Farnsworth, 2001).

In our earlier work(in press), we have observed a significant reduction in parasitaemia when a preparation made from combined extracts of *A. boonei* and *A. senegalensis* is administered to *Plasmodium berghei* parasite infested mice up to a dose of 800mg/kg body weight. The present study is an evaluation of the effect of the herbal preparation on the histopathological damages caused by malaria parasites on the liver of infected mice as futher evidence in support of the observed antiplasmodial effect of the herbal preparation.

## 2. Materials and Methods

### *2.1. 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 senegalensis* were collected from Ihiagwa 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.

### *2.2. 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. senegalensis* were dried under shed for one week. The samples were later milled to powder using a mechanical blander.

### *2.3. 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℃ to yield a gummy residue which was stored in a refrigerator at 4℃

### *2.4. 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.

### *2.5. Malarial Parasites*

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

### *2.6. Inoculation of Parasites*

At the end of the acclimatization period, each of the thirty mice was inoculated with parasitized donor erythrocytes containing about 1 x $10^7$ *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 preparation), Group B (600mg/kg herbal preparation), Group C (800mg/kg herbal preparation), 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, the mice were sacrificed, blood samples taken for measurement of parasitaemia while liver samples were taken for histological studies.

### *2.7. Histological Procedure*

Histological examination was done by fixing the organs (liver) in 4% formaldehyde. They were subsequently processed and embedded in Paraffin wax. Tissue blocks were sectioned 5µm thick and stained with Haematoxylin and Eosin (H & E).

## 3. Results

The liver sections of the animals treated with the different doses of the herbal preparation showed relatively normal histological features similar to that of the animals treated with the standard drug, chloroquine phosphate. On the contrary, liver section taken from the untreated animal group (administered with normal saline) showed severe distortion of the hepatocytes with marked necrosis (Figures 1 – 5).

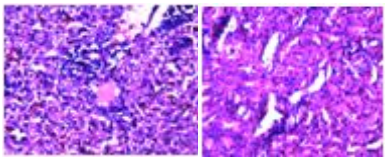

*Fig 1: Photomicrograph of the liver of group A (H & E x 400)*
*Fig 2: Photomicrograph of the liver of group B (H & E x 400).*

*Fig 3: Photomicrograph of the liver of group C (H & E x 400).*
*Fig 4: Photomicrograph of the liver of group D (H & E x 400).*
*Fig 5: Photomicrograph of the liver of group E - Untreated Animals (H & E x 400)*

**4. Discussion**

Our histological findings revealed a severe distortion of the cyto-architecture of the liver parenchyma in the malaria infected but untreated animals thus confirming the injurious effect of malaria on the liver. The other groups of animals equally infected with malaria but treated with the herbal preparation however, showed relatively normal liver histological features similar to that of 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 antimalarial effect

**5. Acknowledgement**

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