

INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213; e-ISSN: 2348-5221)
www.ijrcrps.com



Research Article

EFFECT OF DI-HERBAL MIXTURE OF *ANNONA SQUAMOSA* LINN. AND *ALSTONIA BOONEI* DE WILD ON HISTOPATHOLOGICAL DAMAGES CAUSED BY *PLASMODIUM BERGHEI* IN THE LIVER OF INFECTED ALBINO MICE

¹ONWUSONYE J.C., ²UWAKWE A.A. AND ²PATRICK-IWUANYANWU K.C.

¹Department of Biochemistry, Federal Polytechnic, Nekede-Owerri Imo State, Nigeria.

²Department of Biochemistry, University of Port Harcourt, Rivers State Nigeria.

Corresponding Author: Jconwusonye@gmail.com

Abstract

The effect of di-herbal mixture of *Alstonia boonei* and *Annona Squamosa* (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 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 = 5 each). Group 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, 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 distortion of the hepatocytes with marked necrosis. These findings thus support the combination of the two herbs for higher synergistic antimalarial effect.

Keywords: *Alstonia boonei*, *Annona Squamosa*, histopathological damages, *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 population 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 histopathological damages caused by malaria parasites on the liver 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, the mice were sacrificed, blood samples taken for measurement of parasitaemia while liver samples were taken for histological studies.

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) for detailed observation

Results

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

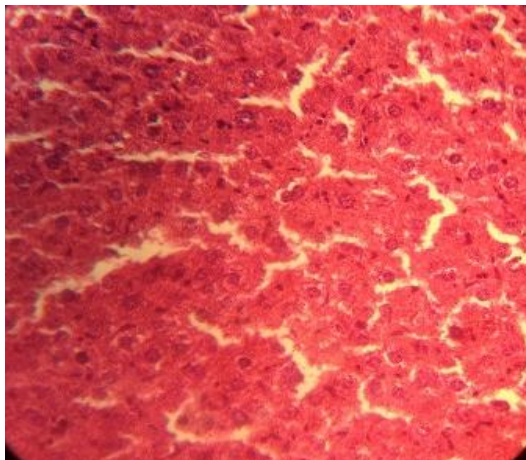


Fig.1: Photomicrograph of Infected liver administered with di- herbal mixture (BN + SL 400mg/kg) showing essentially preserved hepatocytes with slight degree of inflammatory response (H&E x400)

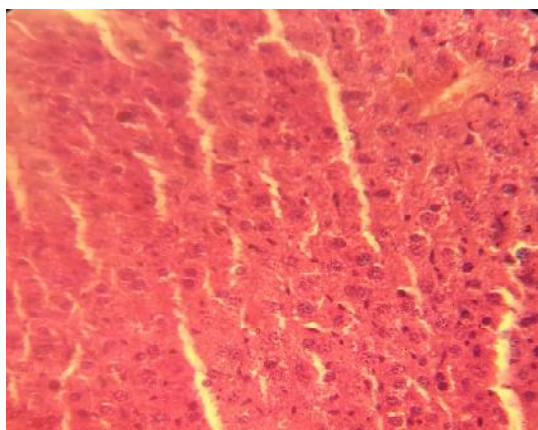


Fig.2: Photomicrograph of Infected liver administered with di- herbal mixture (BN + SL 600mg/kg) showing essentially preserved hepatocytes with slight degree of inflammatory response (H&E x400)

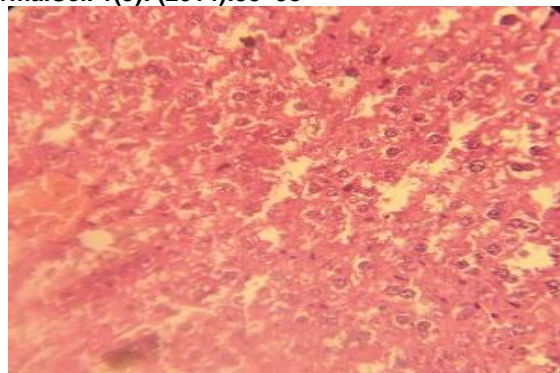


Fig.3: Photomicrograph of Infected liver administered with di- herbal mixture (BN + SL 800mg/kg) showing essentially preserved hepatocytes with slight degree of inflammatory response (H&E x400)

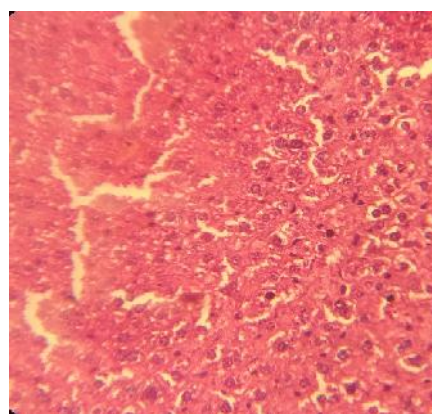


Fig.4: Photomicrograph of Infected liver administered with Chloroquine phosphate (5mg/kg) showing essentially preserved hepatocytes with slight degree of inflammatory response (H&E x400)

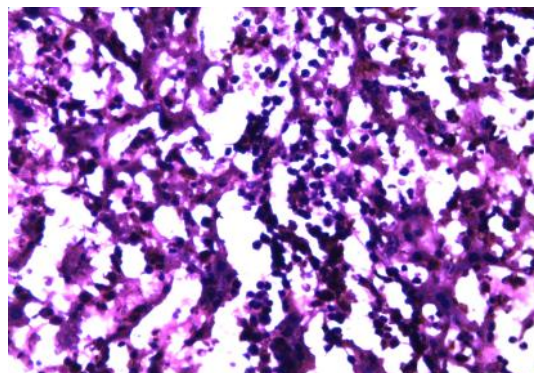


Fig.5: Photomicrograph of infected liver administered with normal saline (5ml/kg) showing severe distortion of hepatocytes with marked necrosis. (H & E x 400).

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 deleterious effect of malaria on the liver. The other groups of animals equally infected with malaria but treated with the herbal mixture 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 synergistic antimalarial effect

Acknowledgments

The authors hereby acknowledge the technical support received from Prof. Omotayo Ebong, Director Step B Malaria Research Centre, University of Port Harcourt.

References

- Idowu O. A., Soniran O. T., Ajana O. and Aworinde D. D. (2010). Ethnobotanical survey of antimalarial plants used in Ogun State, South-West Nigeria. *African Journal of Pharmacy and Pharmacology* 4:055-060.
- Johns T., Windust A., Jurgens T. and Mansor S.M. (2011). Antimalarial alkaloids isolated from *Annona squamosa*. *Phytopharmacology* 1(3) 49-53.
- Kirtikar K.R. and Basu B.D. (1957). Indian Medicinal Plants. 2nd edition vol. 1 pp 66-69
- Oliver, B. D. (1986). Propagation and management, functional uses of medicinal plants in: Medicinal plants, Tropical West Africa. Cambridge University Press pp. 89 – 90.
- Ryley J. F. and Peters W. (1970). The antimalaria activity of some quinolone esters. *Am. Trop. Med Parasitol.*, 84:209-222.
- Soniran O. T., Idowu O. A., Ajayi O. L. and Olubi L. C. (2012). Comparative study on the effects of Chloroquine and Artesunate on Histopathological Damages caused by *Plasmodium berghei* in four vital organs of infected Albino mice. *Malaria Research and Treatment*. Vol. 2012 (2012). 960 758.
- Titanji V. P. K., Zofou D. and Ngemenye M. N. (2006). The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroun Folk Medicine. *African Journal of Traditional Complimentary Alternative Medicine* 5: 302 – 321.
- WHO (1977). Selection of Essential Drugs. Technical Report Series, No. 615 Geneva.