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ANTI-PLASMODIAL REMEDY OF FOUR DIFFERENT BOTANICALS (LEAVES AND STEM EXTRACTS) IN MICE INFECTED WITH *PLASMODIUM BERGHEI*

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ABSTARCT

Azadiracta indica, Carica papaya, Morinda lucida and Alstonia boonei are common medicinal plants used widely in Nigeria for the treatment of malaria and other life threatening ailments. Many efforts are being sacrificed to develop more potent and cheaper antimalarials of plant origin to combat and replace the various antimalarial drugs that no longer resist the effect of plasmodium. This work was set out to investigate the antiplasmodial effect of the four botanical extract in plasmodium berghei infected mice using the Peter's 4-days suppressive test. 25 healthy BALB/c mice of average weight 13.5g were divided into5 groups of 5 mice each. Group 1 was the negative control given 5mg/kg/bw of normal saline solution while group 2-5 were inoculated intraperitoneally plasmodium berghei parasitized red blood cell, followed by daily administration of the four ethanolic extract. Administration of 20, 40, 80, 120, 240 and 300mg/kg/bw of the extract showed significant ($p<0.05$) suppression of plasmodium berghei load in mice infected. Each of the extract was dose dependent as LC_{50} was observed at 80mg/kg/bw for Alstonia boonei, Morinda lucida and Carica papaya and 120mg/kg/bw for Azadiracta indica. The result of this study showed that the four ethanolic leaf and stem extract possesses potent antimalarial effects and may therefore offer a potential drug lead for development of a safe, effective and affordable antimalarial.

Keywords: Anti-plasmodial, *Plasmodium berghei* and Botanical extracts

Introduction

Africa continent is blessed with array of medicinal Plants that have been proved beyond doubt as remedies for wide range of ailments many that are peculiar to tropical and sub-tropical countries. Potentials of many of these medicinal Plants are yet to be elucidated or not yet exhausted. Malaria is one of the tropical diseases that has remain despite every effort ,this can be attributed to numerous factors including ,vector resistance to pesticide, parasite resistance to common available drugs, and high cost of procuring thee effective drugs by the common populace some that suffer the attack mostly. Malaria is believed to be a major obstruction to social and economic development in Africa, causing enormous misery and suffering through the pains of fever s and the anguish of bereavement (Ayoade *et al.,* 2014).

Artemisinin from Artemisia annua has become one of the most important drugs for malaria therapy (Schramek et al.,2010) .Artemisinin –based combination therapies are the bestanti-marial drugs available now.In Nigeria like many other African nation, the disease is usually been treated by self-medication, the use local herbs, use of the services of spiritualists/traditional priests or/and the use of clinic/hospital services (Jimoh *et al.*,2006).*Plasmodium berghei* which causes murine malaria in mice is widely used as a simulation to the studies of human malaria. While it will be out-rightly impossible to culture *P. falciparum* in vivo, *P. berghei* hasthe advantages of ease of propagation. In addition the in vitro cultivation of *P. falciparum* decreases in viability with timeSynthetic pharmaceutical products are not without their own side effects also, these drugs are costly and not at reach of all (Zihiri *et al.,* 2005). In mostAfrican settlements and villages Traditional herb medicine is the most frequent option to treat malaria due to its availability, low cost and reduced side effect (Tiwari *et al*., 2013).

Several authors have studied the antimalarial activities of these plants extracts and had reported their efficacy especially against human malaria. The aim of the current research is comparatively studies of potentiality of some selected plants extracts against the in vivo cultured *Plasmodium berghi* in mice. *A. boonei* in-vivo activities against p bergei in mice has earlier been reported by Olajide *et al.*,2000 and Awe et al.,1990.The anti-plasmodial efficacy of leaves extracts of *Carica Papaya* has been reported by earlier authors(Kovendan *et al.*,2012). Earlier Studies have showed that *Morindalucida* leaves extract appears to have schizontocidal and repository effects in mice infected with *P. berghei Azadiracta indica* as herbal treatment for various ailments including

malaria has been reported widely by various authors and the efficacy has never in doubts (Akin-Osanaiye *et al*., 2013).The studies aim to elucidate and compare the anti-plasmodial effects of the extracts of the three plants.

Materials and Method

Fresh stem bark of *Alstonia boonei, Carica papaya leaves, Morinda lucida stem barkand Azadiracta indica* stem bark were all collected within Ilaro Township and suburb. The plants were identified by a Departmental Plant taxonomist and adequately numbered for future references.

Preparation of plant extract

The fresh stem bark was properly cleaned using clean water, cut into pieces and was air dried under shade for 1 month. The dried stem bark was reduced to powder by first grounding to coarse powder in a mortar then blended to almost fine powder using a giant electric blender. Extraction was carried out by dispersing 300g of the grounded plant material in 1.5L 95% ethanol and was shaken manually for some time after which it was left to stand overnight in the laboratory. The extract was filtered using filter paper then the filtrate (extract) was then concentrated using a rotary evaporator. The concentrate was heated over a water both to obtain a solvent free extract which was store in the fridge for the work.

This procedure of extraction was separately carried out on other specimens of *Carica papaya leaves, Morinda lucida (stem bark), Azadiracta indica (leaves), Azadiracta indica (stem bark) as well as Azadiracta indica (root).*

Phytochemical analysis for bioactive constituents both qualitative and quantitative were carried out on the ethanolic extract, aqueous extracts and the dried powdered of all the test plants using standard procedures as described by Trease and Evans (1989), Harborne (1973) and Sofowora (1993).

Experimental Animals:

Sixty Balb/cmice of eight week old with average weight of 13.5g were used in this study. They were obtained from Institute for Medical Research and Trainee (IMRAT), University College Hospital (UCH), Ibadan, Nigeria. They were housed in standard Plastic cage with saw dust as

Litters. Normal pellet feed formulation and water was given and the mice were used in accordance with NIH guide for the care and use of laboratory.

Animal grouping for inoculation and treatment:

The parasite *Plasmodium berghei* was obtained from Nigerian Institute for Medical Research (NIMR), Yaba, Lagos, Nigeria. The parasites were kept alive by infecting two mice which were fed well to be alive (donor mice).These infected mice were kept and observed to reproduce disease symptom. The remaining mice were randomly divided into five (5) groups with each group containing five mice.

The parasitaemia level of the donor mice were first determined by cutting the tail of the animal with a sterile pair of scissor, and a smear of the blood was made on a slide which thereafter was observed under the microscope using the oil immersion magnifying lens to show the presence of parasite.

Since the parasitamia level of the donor mice was observed to be high, 0.7ml of blood was collected from donor mice and diluted with 27ml of normal saline such that 0.2 ml contain standard innoculum of $1x10^7$ infected red blood cell (Maegraith *et al*., 1952).

Twenty acclimatizes mice were inoculated intraperitoneally from the same source to avoid variability in parasitemia.The mice were randomly distributed into four groups with five mice per group. The four groups of infected mice were administered 20, 40, 80, 120, 240 and 300mg/kg/day of plant extract, and 5mg/kg/day of normal saline (the control group) for four consecutive days (following Peter's 4-days suppression test against *P. berghei* infected in mice (Peter, 1970).

Blood was collected from the tail of each mouse each day and smear on a slide to make a thin film. The blood film was fixed with methanol, stained with Giemsa stain for 20mins, rinsed with little water and then air dried. The dried slide was observed under microscope for the presence of parasites. The %suppression of parasitaemia was calculated in comparison with the negative control.

RESULTS AND DISCUSSION

Table 1: Phytochemical contents of selected Plants

Phytochemical constituents	Plants			
	A. boonei	**B. papaya**	**M. lucida**	**A. indica (Stem bark)**
Alkaloids	+	+	+	+
Tannin	+	+	+	+
Phlobatanin	-	-	-	-
Flavonoid	+	+	+	-
Steroid	+	+	+	+
Saponin	+	+	+	+
Cardiac glycoside	-	+	+	-
Terpenoid	+	+	+	+

+ =Present

- = absent

Table 2: Parasitemia *Estimation* at different concentrations of the extract

Dose (mg/kg/bw)	Average % Parasitaemia caused by various plants			
	A.boonei	***C.papaya***	***M.lucida***	***A.indica***
0	51.5 ±0.02	65.1 ±0.01	40.3±0.03	56.0±0.03
20	45.3±0.05	58.0 ±0.02	35.5±0.02	38.8 ±0.02
40	36.2±0.02	53.7 ±0.04	30.2±0.01	24.9 ±0.01
80	23.3±0.02	32.8 ±0.02	22.4±0.03	18.1 ± 0.02
120	20.8±0.01	10.6 ±0.04	20.2±0.02	10.3 ± 0.01
240	13.3±0.02	Death	17.3±0.02	Death
300	Death	-	Death	-

Results were means of three replicates ±SEM

Table 3: Approximate Lethal concentration of the Extracts

	Plant Extracts			
Lethal Conc.	*A.indica*/mg/kg/bw	*A.boonei*/mg/kg/bw	*C.papaya*/mg/kg/bw	*M.lucida*/mg/kg/bw
Lc_{50}	120	80	80	80
Lc_{75}	240	120	120	120
Lc_{100}	300	240	240	240

The existence of experimental animal models of a disease aids not only in the understanding of the patho-physiology of such disease, but also in the development of drug candidate (Okpe *et al.,* 2012). Therefore, screening for antimalarial activity of plant crude extracts is the first step in isolation of new molecules with potent activity (Ma *et al.,* and Njoroge *et al.* 2006).

The effect of the ethanolic crude extract of the stem bark of *Morinda lucida*, *Alstonia boonei, Azadiracta indica* and the leaves of *Carica papaya* on *Plasmodium berghei* are presented in table 2 and 3. All the extracts exhibited antimalarial activity against *Plasmodium berghei* in a concentration dependent manner.The efficacy of the ethanolic extracts of the four test plants in suppression of parasitemia load in *Plasmodium berghei* infected mice were reported by earlier workers (Usha *et al.,* 2001; Fakoya *et al.,* 2011; Iyiola *et al.,* 2011 and Oche *et al.,* 2016). The significant chemo suppression is also in agreement with the traditional use of the plant as an herbal medication against malaria in many parts of Nigeria.

It is shown in table 2 above that there is significant ($p<0.05$) variation among the *Plasmodium* infected and treated groups before the administration of any of the botanical extracts. The variation could be due to the physiological state of each group of mice as corroborated by the work of (Jenkins and Facer, 1985 and Stephen, 1986).Further disparity in the infected and treated groups may be as a result of the difference in efficacy of each of the botanical extract in their chemo-suppression ability.

It is indicated in table 3 the lethal concentration of each of the extracts.Each of the test plant is dose dependent. Our result shows that the parasitemia load vary significantly ($p<0.05$) in all the groups administered with the various botanical extract as LC_{50} was observed at 80mg/kg/bw for *Alstonia boonei, Carica papaya and Morinda lucida* extract and 120mg/kg/bw for *Azadiracta indica*. LC_{100} was observed at 240mg/kg/bw for *Alstonia boonei, Carica papaya* and *Morinda lucida* and 300mg/kg/bw for *Azadiracta indica*. This shows that dose less than 80mg/kg/bw of *Alstonia boonei, Carica papaya* and *Morinda lucida* and less than 120mg/kg/bw of *Azadiracta indica* is tolerable by the animal and can effectively suppress plasmodium load in infected animals.

The experiment clearly indicate that the individual administration of aqueous stem bark extract of *Alstonia boonei, Morinda lucida, Azadiracta indica* and leaf extract of *Carica papaya* plants significantly ($p<0.05$) decreased *Plasmodium berghei* load in mice and enhanced their survival. There are many bioactive constituents present in these extracts as shown in table 1. The antimalarial activity of the extracts could be attributed to these phytochemicals present in the plants used, but specifically, it could be attributed to the alkaloids present in all the four plantextract as reported in past work of (Adebayo and Kretli, 2011).

However, some other reports have shown that flavonoids, tannins, saponins, and other phyto constituents may play vital roles in the inhibition of malaria parasites in infected animals (Igile *et al.*, 1994; Udensi *et al.*, 2002)

CONCLUSION

It is clear from the results obtained in this work that in *Plasmodium berghei* infected mice treated with extracts of *Alstonia boonei, Azadiracta indica, Morinda lucida* stem bark and *Carica papaya* leaf, the percentage of parasitaemia measured reduced significantly ($p<0.05$). The result presented herein suggest that the ethanolic stem extract of *Alstonia boonei, Azadiracta indica, Morinda lucida* and the leaf extract of Carica papaya is safe and possess very potent anti-malarial activity and this clearly justifies their use as traditional medicine for combating plasmodium infection.

REFERENCES

Adebayo, J.O. and Krettli, A.U. 2011. Potential antimalarials from Nigerian plants: *A review Journal of Ethnopharmacology.*2011;133:289–302. doi: 10.1016/J.JEP.2010.11.024.

Akin-Osanaiye, B.C., Nok, A.J., Ibrahim, S., Inuwa, H.M., Onyike, E., Amlabu, E. and Haruna, E. 2013. Antimalarial Effect of Neem Leaf and Neem Stem Bark Extracts on Plasmodium Berghei Infected In the Pathology and Treatment of Malaria.*International Journal of Research in Biochemistry and Biophysics.*

Fakoya, A.1., Owojuyigbe, O.S., Fakoya, S. and Adeoye, S.O. 2011. Possible antimicrobial activity of *Morinda lucida* stem bark, leaf and root extracts. *African Journal of Biotechnology,* Vol 13(3), pp. 471-475.

Igile, G.O., Oleszek, W.,Jurzysta, M., Burda, S., Fafunso, M. and Fasanmade, A.A. 1994. "Flavonoids from *Vernonia amygdalina* and their antioxidant activities," *Journal of Agricultural and FoodChemistry*, vol. 42, no. 11, pp. 2445–2448, 1994.

Iyiola, O.A., Tijani, A.Y. and Lateef, K.M. 2001. Antimalarial activity of ethanolic stem bark extract of Alstonia boonei in mice.*Asian Journal of Biological Sciences.* 2011;4:235–243. doi: 10.3923/ajbs.2011.235.243

Jenkins, G.C. and C.A. Facer, 1985. Hematology of African Typanosomiasis. In: Immunology and Pathogenesis of Trypanosomiasis, Tizard, I. (Ed.). CRC Press, Boca Raton, Florida, pp: 13-44

Ma, C., Zhang, H.J. and Tan, G.T. 2006. "Antimalarial compounds from *Grewia bilamellata*," *Journal of Natural Products*, vol. 69, no. 3, pp. 346–350, 2006.

Njoroge, G.N. and Bussmann, R.W. 2006. "Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya)," *Journal of Ethnobiology andEthnomedicine*, vol. 2, article 8, 2006.

Oche, O., Nathan, H., Joseph, I., Vincent, A.U., Stanley, I.R.O. and Omiagocho, T.I. 2016. Antimalarial potential of Carica Papaya and Vernonia amygdalina in Mice Infected with Plasmodium berghei. *Journal of Tropical Medicine.*

Okpe, O., Abdullahi, A., Ihuoma, S.O., Nkeonye, O.L., Ilechukwu, C and Nweke, O. 2012. "Hypoglycaemic and hypolipidaemic effects of ethanolic and aqueous leaf extract of *Vitex*

doniana in normoglycemic albino rats," *Global Advanced ResearchJournal of Microbiology*, vol. 1, no. 10, pp. 173–179, 2012.

Stephen, L.E. 1986. Trypanosomiasis: A Veterinary Perspective. Pergamon Press, New York.

Udensi, E., Ijeh, I. and Ogbonna, U. 2002. "Effect of traditional processing on the phytochemical and nutrient composition of some local Nigerian leafy vegetables," *Journal of Science andTechnology*, vol. 8, pp. 37–40, 2002.

Usha D. 2001. Screening of Natural/Synthetic Compounds for Antimalarial Activity. 25 years of Malaria Research Centre. A Profile of National Institute of Malaria Research Parasite Biology. 77-81.