Scientia Africana, Vol. 18 (No. 3), December, 2019. Pp 1-6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

POSSIBLE ANTI MALARIA POTENTIAL OF NWAGBAKELU FORMULATION AND ITS EFFECT ON SELECTED LIVER FUNCTION INDICES OF ALBINO MICE

[1]Eucharia Oluchi Nwaichi[*], [2]Justice Obinna Osuoha and [3]Blessing Onyinye Orakwute

[1,2,3]Department of Biochemistry, University of Port Harcourt, Choba, Rivers State, Nigeria
*Correspondence
nodullm@yahoo.com

Received: 08-08-19
Accepted: 02-09-19

ABSTRACT

The anti-malarial potential of a locally formulated trado-medicine, Nwagbakelu and its effects on selected liver function parameters was investigated on malaria infected albino mice. Study mice were grouped into A, B, C; Group A mice was administered colart 0.1mL; Group B mice was not treated and it served as negative control; Group C mice were treated with nwagbakelu. All treatment lasted for five (5) days, mimicking recommended administration pattern. The activity of a standard malaria drug Colart which is a combination of artemether and lumenfantrine was used as a positive control for comparison. In comparison to the positive control samples a 100 % elevation of alanine aminotransferase (ALT) was observed in nwagbakelu treated mice whilst the non-treated and Colart treated mice maintained elevated level of blood concentration of aspartate aminotransferase (AST), alkaline phosphatise (ALP), total protein, albumin, and conjugated bilirubin. The percentage parasitemia of each group were obtained everyday of treatment. No significant differences were found for percentage parasitemia in group B mice for the study period, while those of those of group A showed marked 100 % clearance in all mice but one mice which was mildly (4%) infected the end of day 5. Nwagbakelu cleared the parasite completely at percentage parasitemia of 12 and reduced the parasitemia of other infected mice by 8% daily. These findings highlight the anti-malarial potentials of nwagbakelu and raise concerns on possible hepatotoxicity of this herbal formulation by virtue of elevation of ALT.

Keywords: Anti-malaria; Herbal formulation; Nwagbakelu; Medicinal plants; Hepatotoxicity

INTRODUCTION

According to World Health Organization, (2017) malaria accounts for approximately half a million deaths yearly and over 210 million clinical trials. It is even more problematic because almost half of the world's population is at risk of malaria. Malaria is prevalent in Africa and Nigeria specifically accounts for ¼ of all clinical malaria cases in Africa (WHO, 2008).

Reports from the Federal Ministry of Health (2004) in Nigeria reveal that malaria is the prevalent disease in the country and more than half of their populaces are predisposed to this disease annually (Adebayoa and Krettli, 2011). In humans, four different parasite species of plasmodium genus has

been documented to perpetuate malaria. They manifest from one individual to another through a bite from female anopheles mosquito (Odoh *et al.*, 2018).

In most endemic areas, plasmodium parasites are developing growing resistance to existing anti-malaria drugs (WHO, 2011), as well as the artemisinin-based combinations (Htut, 2009; Cui *et al.*, 2012). If care is not taken, there are concerns that a total resistance to orthodox drugs may be developed by the parasite (Dike *et al.*, 2012). Since the results of most chemoprophylactic agents haven't been completely favorable, these concerns are now more troublesome (Ntie-Kang *et al.*, 2014). Therefore, there is fervent search for safer and more efficient drugs that will help to combat this disease successfully.

The application of herbal formulations as substitutes for orthodox medicine is gaining remarkable recognition because of availability, cost and accessibility all over the world (Nwaichi and Osuoha, 2018). Medicinal plants have been utilized for the treatment of malaria traditionally for decades. There exists some strong evidence of more than 1100 plant species from diverse families utilized in the treatment of fever or malaria (Willcox and Bodeker, 2004). Besides, the two most thriving anti-malarial drugs (quinine and artemisinin) are of plant origin (Willcox and Bodeker, 2004; Batista *et al.*, 2009).

Nwagbakelu is a herbal combination of six plants different plants; palm leaves, gmelina leaves, guava leaves, lime fruits, pawpaw leaves and pineapple peel that is commonly utilized by the indigenous people of Anambra State in Nigeria for the treatment of malaria. There is paucity of information regarding the potency and safety of this herbal formulation. In view of this, it is expedient to ascertain the efficacy of this herbal formulation in order to underscore the anti-malaria capability.

MATERIALS AND METHODS

Sample collection

The palm leaves, gmelina leaves, guava leaves, lime fruits, pawpaw leaves and pineapple peel used for the preparation of Nwagbakelu were obtained within the premises of University of Port Harcourt. These plant samples were authenticated at the Plant Science and Biotechnology department, University of Port Harcourt.

Sample preparation

The obtained samples were washed in clean water with their stems. *Ananas comosus* was washed in clean water processed to obtain the peel and pulp. The aurantifolia was washed in clean water and then cut into two equal parts. The clean samples (90 grams each) plus two aurantifolia fruits were mixed together in a cooking pot with 1 litre of water and then boiled for 25 mins. The resultant formulation was allowed to cool prior to administration.

Preparation of standard drug (Colart)

Colart is anti-malaria drug that has combination of 80g of Artemether and 120g of Lumefantrin. The concentration of the drug Colart was prepared based on the bodyweight of the mice. Calculations was done using the average bodyweight of human as 35kg (35000g) to deduce the concentration of drug required for the mice according to their various weights. Based on calculations, four tablets of colart were dissolved in 100ml of distilled water, and

Scientia Africana, Vol. 18 (No. 3), December, 2019. Pp 1-6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

0.1 ml of the drug solution was administered orally to the positive control (Group A) only using a 1 ml syringe in the morning and evening for three consecutive days.

Experimental Animals

Fifteen Wistar mice were used for this experiment. The mice were obtained from the animal farm of the Department of Animal and Environmental Biology, University of Port Harcourt. Three equal groups were created from these animals and they were permitted to acclimatize for one week. They mice' were sustained on a commercial mash and water *ad libitum* throughout the experiment.

Inoculation of mice

For the inoculation of mice with plasmodium bergheri, the parasites were maintained in the laboratory by the method described by Bruce, (1980). The mice were inoculated intraperitoneally with 0.2 ml of parasitized saline suspension containing 6 X 10^7 parasites. Parasitemia development was monitored by microscopic examination after 120 h (5 days) to obtain high parasite infection.

Estimation of parasite level in the inoculated mice

The blood samples were collected from the tip of the mice tail as described by UCI (2015) in the morning of the fifth day of the inoculation to determine the parasitemia level before commencement of treatment. The parasite count for each blood film was examined under oil immersion using tally counter.

Method of treatment

Group A rats were treated with 0.1 ml of standard drug Colart twice (morning and night) daily for the three days and they served as the positive control

Group B rats were not treated but received normal feed and water for eight days after inoculation and served as the negative control

Group C rats were treated with 0.2ml of Nwagbakelu twice (morning and night) daily for the three days.

Collection and preparation of blood samples

The mice were intubated using chloroform and deep incisions were made to locate the heart for cardiac puncture to obtain the blood sample. The blood sample was collected with lithium-heparin bottles. The blood was spun with a centrifuge and the supernatant serum was collected and used for biochemical analysis.

Determination of biochemical indices

The serum concentration of ALP, ALT, AST, total protein, albumin and bilirubin were determined enzymatically using Randox Kits. All samples were analyzed in triplicates.

RESULTS

The results of the observed % parasitemia is as shown in Tables 1 to 3.

Table 1: Percentage parasitemia of mice in group A (positive control i.e. infected with parasite and treated with standard dug)

	Day 1	Day 2	Day 3	Day 4	Day 5
1	46	19	7	3	No parasite seen
2	50	25	19	7	2
3	36	14	7	3	No parasite seen
4	45	17	9	2	No parasite seen
5	20	7	3	No parasite seen	No parasite seen

Table 2: Percentage parasitemia of mice in group B (negative control i.e. infected with parasite but not treated)

Mice	Day 1	Day 2	Day 3	Day 4	Day 5
1	50	62	69	72	74
2	47	53	56	69	71
3	19	27	52	64	69
4	24	30	41	53	58
5	45	50	55	67	70

Table 3: Percentage parasitemia of mice in group C (group infected with parasite and treated with Nwagbakelu formulation)

Mice	Day 1	Day 2	Day 3	Day 4	Day 5
1	45	37	23	12	5
2	45	23	16	Dead	Dead
3	12	5	2	No parasite seen	No parasite seen
4	25	16	7	6	4
5	56	48	30	23	Dead

Table 4: Effect of the treatment on selected liver function markers

Groups	AST	ALT	ALP	T.P	ALB	T.B	C.B
A	40.6	4	116.8	62.4	32.6	9.2	5.34
B	31.8	4.8	126.4	65.4	30.8	9.64	5.32
C	24.7	8	98	63.3	28	8	4.0

n=5 for group A and B, n =3 for group C (as 2 mice died prior to sacrifice – see Table 3)

DISCUSSION

The prevalence of multiple drug resistance had led to the fervent search of cheaper, safer and more efficient drugs that will help combat malaria disease in most developing countries. Majority of the general population in Nigeria and other developing countries subscribe to the use of herbal medicines and formulation (Nwaichi and Osuoha, 2018).

In this study, the percentage parasitemia of the wistar mice inoculated with the malaria parasite were obtained daily in order to determine possible progress in treatment. As demonstrated in (Table 2), the daily percentage parasitemia of the mice in group

Scientia Africana, Vol. 18 (No. 3), December, 2019. Pp 1-6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

B (negative control) increased daily because the mice were not treated after inoculation. However, results obtained for the mice in group A (positive control) treated with a popular anti-malaria colart, which is a combination of artemether and lumenfantrine revealed a significant decrease from the day 1 through day 4 and no parasite was observed on the fifth day which is an indication of total recovery. Interestingly, only one mouse in group 3 treated with the herbal formulation "nwagbakelu" demonstrated a total recovery in the fourth day of treatment with no parasitemia found. However, other mice in the same goup (Table 3) treated with the formulation revealed a duration dependent decrease in the parasitemia load like those in group A. But the parasitemia loads were still present even on the fifth day which is an indication that the herbal formulation may require additional day of treatment in order to ascertain satisfactory levels of parasitemia clearance and total recovery.

Furthermore, the liver is one of the essential organs in the animal body because it is mainly the site of eradication and deactivation of certain toxic xenobiotics (Adeoti *et al*., 2017). Based on this, it is pertinent to ensure the integrity of the liver is not compromised with drugs or any xenobiotic introduced into the body. Results from the liver function markers (Table 4) in this study reveal that only the concentration of aspartate aminotransferase (ALT) in the group treated with the herbal formulation "nwagbakelu" was elevated when compared to other groups. Aspatate aminotransferase and alanine transferase are sensitive indicators for any form of hepatic injury (Barth, 1979) and they are known to reorganize proteins' building blocks (Nwaichi *et al*., 2017). Thus, high concentrations of these enzymes in the plasma are clear indication of possible hepatotoxicity (Konan *et al*., 2007). So the high levels of aspartate aminotransferase observed in the group treated with the herbal formulation indicate that the formulation may contain toxic compounds that have interfered with the safety of the hepatocytes of the mice in that group.

CONCLUSION

The herbal formulation "nwagbakelu" exhibited potent anti-malaria activity on the malaria infected Wistar albino mice. However, caution should be applied when using this herbal formulation because it can cause liver damage by virtue of elevation of ALT.

Conflict of Interest

The authors declare no conflict of interest regarding this article.

REFERENCES

Adeoti, O. T., Belonwu, D. C., Wegwu M. O., and Osuoha, J. O. 2017. Implication of acute, sub-chronic and chronic exposure to different pesticides via inhalation on male Wistar rats, Bioengineering and Bioscience, 5 (4), 74-85. Doi:10.13189/bb.2017.050403

Barth, P. T. (1979). RP4 and R300B as wide host-range plasmid cloning vehicles. Plasmids of medical, environmental and commercial importance, 1: 399-410.

Batista, R., de Jesus, A., Júnior, S., de Oliveira, A.B., 2009. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. Molecules 14, 3037–3072.

Bruce, C. L. J. (1980). Essential malariology. London, Willian Heinemann Medical books Ltd, London, pp 102-113.

Cui, L., Wang, Z., Miao, J., Miao, M., Chandra, R., Jiang, H., Su, X.Z., Cui, L., 2012. Mechanisms of in vitro resistance to dihydroartemisinin in Plasmodium falciparum. Mol. Biol. 86 (1), 111–128.

Dike, I.P., Obembe, O.O., Adebiyi, F.E., 2012. Ethnobotanical survey for potential antimalarial plants in south-western Nigeria. J. Ethnopharmacol. 144 (3), 618–626.

Federal Ministry of Health, 2004. National Antimalarial Treatment Policy. Federal Ministry of Health, Abuja, Nigeria.

Htut, Z.W., 2009. Artemisinin resistance in Plasmodium falciparum malaria. New Engl. J. Med. 361, 1807–1808.

Konan, N. A., Bacchi, E. M., Lincopan, N., Varela, S. D., and Varanda, E. A. (2007). Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (Anacardium occidentale L.) *Journal of ethnopharmacology*, 110(1): 30-38.

Ntie-Kang, F., Onguéné, P.A., Lifongo, L.L., Ndom, J.C., Sippl, W., Mbaze, L.M., 2014. The potential of anti-malarial compounds derived from African medicinal plants, Part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malar. J. 13, 81.

Nwaichi EO, Osuoha JO (2018) Essential Oil and Fatty Acid Constituents of *Buccholzia coriacea* (Wonderful Kola) Seeds Harvested in Nigeria. Biochem Physiol 7: 233. doi: 10.4172/2168-9652.1000233

Nwaichi, E. O. Osuoha, J. O. and Monanu, M. O. Nutraceutical Potential of Tetracarpidium conophorum and Buccholzia coriacea in Diet-induced Hyperlipidemia. *Journal of Chemical Health Risks*, 2017; 7 (3), 157–170.

Odoha, U. E., Uzorb, P. F., Ezea, C. L., Akunnec, T. C., Onyegbulama, C. M., Osadebeb, P. O. 2018. Medicinal plants used by the people of Nsukka Local Government Area, south-eastern Nigeria for the treatment of malaria: An ethnobotanical survey. Journal of Ethnopharmacology, 218, 1–15.

WHO, 2008. World Malaria Report 2008. World Health Organization, Geneva, pp. 7–15 (99–101).

WHO, 2011. World Malaria Report 2011. World Health Organization, Geneva.

Willcox, M.L., Bodeker, G., 2004. Traditional herbal medicines for malaria. Br. Med. J. 329, 1156–1159.

World Health Organisation, 2017. WHO World malaria report. In: WHO [Internet]. 2017 [cited 11 Feb 2018]. Available. http://www.who.int/malaria/publications/ world-malaria-report-2017/report/en/.