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Journal

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JOURNAL OF MALARIA AND PHYTOMEDICINE
(The official Journal of the Centre for Malaria Research and Phytomedicine)

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Introduction

Keep the introduction short. The basic principles of research, background, earlier work, justification and the purpose of the present studies should be described in the introduction.

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The results should be presented in the form of tables or figures when applicable. Sufficient data, all with evidence of statistical analysis, should be presented so that the reader can interpret the results of the experiment.

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The discussion should interpret the results clearly and not repeat the results already stated. It should be concise in terms of biological mechanisms and significance and also should integrate the research findings with the body of previously published literature to provide the reader with a broad base on which to accept or reject the hypotheses tested. Extensive discussion of relevant literature is within bounds. Explain how the results relate previous findings, either in support, contradiction, or simply as added data. Highlight the significant/unique findings of the research under conclusion, and give some Recommendations.

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Books

Clarke N. Nutrient Requirements of Dairy Cattle. 6th Rev. Edn.Natl.Acad. Press, Washington, D.C; 1989. pp: 90-110. A Chapter in a Book: Leach J. Impacts of Dreissenapolymorpha on Water Quality and Fish Spawning Reefs of Western Lake Erie. In: Zebra Mussels: Biology, Impacts and Control. Nalepa, T. and D. Schloesser (Eds.). Ann Arbor, MI: Lewis Publishers, 1993. p: 381-397.

A Report

Makarewicz, J.C., T. Lewis and P. Bertram, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992.U.S. EPA Great Lakes National Program, Chicago, IL.EPA 905-R-95-009. Conference/Proceedings: Muhammad, B.F. and R. Kwali, 2005. Prospects and constraints to small scale yoghurt production in Bauchi Metropolis. Proceeding of the 10th Annual Conference of the Animal Science Association of Nigeria. University of Ado-Ekiti, Sep. 12-15, pp: 234-236.

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Editorial
War Against Malaria

…and the battle goes on;
The humming in our ears
The blood stains on our pillows
The bumps on our skin
The fever and pains that follow thereafter
And of course,
the battle.
Stop the fever
Stop the headache
Stop the joint pains-
Restore the vitality, vibrancy, vivaciousness to our bodies.
But who is the war against?
Hmmm-
I hear a whispering…
The invisible Plasmodium injected by our enemy Mrs Anopheles;
The challenges are apparent but still,
We are resilient
We are determined to win.
Hmmm-
I hear another whispering…
Drug resistance
Yet we fight on,
We continue to march.
And so we invite all stakeholders to come on board to fight this war through the various avenues;
Vector control,
Drug manufacture,
Diagnosis and treatment,
Research,
and of course,
PUBLICATION.
Today we serve you the second volume of our journal;
Enjoy it while we look forward to further contributions.

God bless you all!

Ifeyinwa Chijioke-Nwauche. Ph.D
Editor-in-Chief

Antiplasmodial activity of the lipophilic and hydrophillic fractions of *Pleurotus ostreatus* (Jacq. Ex. Fr) P. Kumm. on *Plasmodium berghei* infected mice model

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ABSTRACT

Introduction:
As a follow-up to our earlier report on the *in vitro* plasmodium lactate dehydrogenase (pLDH) inhibition activity of the edible mushroom *Pleurotus ostreatus*, this present study is reporting the *in vivo* anti-plasmodial activity of the lipophilic and hydrophilic fractions from this edible mushroom.

Methodology:
Freshly collected *P. ostreatus* (5 kg) was cold macerated in absolute ethanol for 72 hours. The ethanol extract was concentrated *in vacuo* to one-tenth and partitioned with dichloromethane giving the lipophilic LF (dichloromethane) and hydrophilic HF (aqueous) fractions. Lorke's method was used for the acute toxicity evaluation of LF and HF. Anti-plasmodial assay was done *in vivo* using the Rane's four days suppressive model on chloroquine sensitive *Plasmodium berghei* (ANKA strain) infected mice with chloroquine as standard control drug. Phytochemical analysis was by using standard methods. Student's t-test of significance ($p< 0.05$) was used for data analysis.

Results:
The LF (yield 0.46 % w/w ; LD_{50} > 5000 mg/kg bw; supressive activity (10 and 1000 mg/kg bw): 71.69 % and 70.91 %) and HF (yield 0.41 % w/w; LD_{50} > 5000 mg/kg bw; supressive activity (10 and 1000 mg/kg bw): 69.3 % and 70.4 %) were both non-toxic and significantly ($p <0.5$) suppresive *in vivo* compared to the untreated group though not as active when compared to chloroquine (100 % suppresion). Mice treated with the higher dose of the HF, showed lesser weight reduction and higher survival rate compared to the lower dose. Phytochemicals: (LF:Fixed oils and triterpenoids and HF :amino acids, and carbohydrates) were present with saponins, phenolics compounds, cyanogenic glycosides, anthraquinones and alkaloids absent in both fractions.

Conclusion:
This observed *in vivo* antiplasmodial activity corroborated the earlier reported *in vitro* pLDH activity of this edible mushroom and is a further evidence of its nutraceutical potential in the management of malaria.

Keywords:
Pleurotus ostreatus, nutraceuticals, malaria, triterpenoids, amino acids, carbohydrates.

INTRODUCTION

The global burden of malaria infections and associated complications have continued to be a challenge for countries in Sub-Saharan Africa. Mostly affected are the poor people, children and expectant mothers in endemic countries with associated high mortality if not treated promptly[1]. Drug resistant *Plasmodium falciparum* strains of the causative parasite in addition to limited access to quality health care facilities and the high cost of orthodox drugs are obstacles necessitating the need for a continued search for new and relatively non-toxic anti-malarial agents. Edible mushrooms are patronized for their nutritive value and functional uses. The anti-malarial[2-3], amoebicidal[4] anti-trypanosomiasis[5] nematicidal[6], anti-inflammatory and immunomodulatory[7] and anticancer[8-9] properties of some edible mushrooms have been documented. As a follow up to earlier reports on the *in vitro* anti-plasmodial activity of *Pleurotus ostreatus*[10] a.bioactive chemical entities bioprospection and nutraceutical potentials of edible Nigerian mushrooms and related mycoflora[11-14] ,this present study is aimed at the

evaluation of the *in vivo* anti-plasmodial activity of *Pleurotus ostreatus* using *Plasmodium berghei* infected experimental mice model.

MATERIALS AND METHODS

Collection of mushroom sample

Pleurotus ostreatus (Fresh fruiting bodies) were collected from the Dilomat farm, Rivers State University of Science and Technology (RSUST), Port Harcourt, Rivers State and identified by a Mycologist in the Department of Crop and Soil Sciences, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State. After due authentication, a voucher specimen (UPH/C/075) was deposited at the herbarium of the Department of Plant Science and Biotechnology of the same University. The fresh fruiting bodies of *Pleurotus ostreatus* were chopped into small pieces after which they were dried under a current of air in a de-humidified environment. The dried samples were pulverized using an electric blender.

Experimental Animals

A total of sixty-six experimental albino mice (25±3g) of both sexes purchased from the animal house of the Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria were used for this study. Due animal handling and ethics guideline were followed. The animals were kept and acclimatized for two weeks in cages, maintained at room temperature under 12 hours dark and light cycle in the animal house and fed with standard diet and portable water *ad libitum*.

Preparation of extract

The freshly collected fruiting body (5 kg) was quenched and cold macerated for 72 hours in absolute ethanol with fresh replacement of solvent at 24 hours interval to obtain the ethanol extract which was concentrated to about one-tenth its volume using a rotary evaporator and subsequently partitioned with dichloromethane to obtain the lipophillic (dichloromethane) fraction (LF) and the polar (aqueous) fraction (HF) used for this study.

Acute Toxicity

This was conducted in a staircase method on experimental albino mice(25-28 g) as reported by Lorke[15] . Briefly, the mice were separately divided into six groups of three mice each according to their body weight. On the first day of the test, the mice in groups 1 to 3 were given 10, 100, and 1000 mg/kg body weight of the test lipophilic fraction LF by oral route using oral gavages to ensure safe ingestion of the preparation and observed for signs of toxicity. After twenty four hours, the remaining three groups were given 1600, 2900 and 5000 mg/kg body weight. The same procedure was done for the hydrophilic fraction HF.

In vivo antiplasmodial evaluation

The Peters' 4-day suppressive test against *P. berghei* infected mice was employed with slight modification[16]. Briefly, adult Swiss albino mice weighing (25-28g) were divided into six groups (A-G) of five mice each per cage and were inoculated by intra-peritoneal (i.p.) injection with 0.1 ml of a diluted blood (in normal saline) containing 2 x10^7 parasitized erythrocytes. The mice were then treated for four (4) consecutive days with daily doses of the fractions, chloroquine or the vehicle by oral route as follows: Mice in groups A and B were respectively treated with 1000, and 10 mg/kg bw of LF, mice in groups C and D were respectively treated with 1000, and 10 mg/kg bw of HF, mice in group E (standard drug control group) were treated with 10 mg/kg bw chloroquine, mice in F (DMSO negative control for LF) and group G (distilled water negative control for HF) were treated with 0.1 ml of DMSO and distilled water respectively.. On day five (5) of the test, thin blood smears were prepared and blood films were fixed with methanol and the blood films stained with Giemsa, followed by examination under the microscope(1000 x magnification). The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Mortality was also monitored daily and throughout the period of study and the percentage survival time determined. Also monitored during the period of study was the change in body weight of each mouse using a sensitive electric weighing balance and the percentage change in body weight of the test samples treated groups compared to the control was determined. All data expressed as mean ± standard deviation, were analysis using the student's t-test of significance ($p < 0.05$)

Phytochemical screening:

This was done using standard phytochemical screening reagents[17-18]. Phytochemical constituents screened for include: alkaloids, phenolics, saponins, cyanogenic

glycosides, anthraquinones, triterpenoids, carbohydrates, amino acids and fixed oils.

RESULTS

The yield of the LF and HF were 0.46 and 0.41 % w/w. Both exhibited an LD_{50} > 5000 mg/kg bw (see Table 1). Table 2 showed the result of the four day suppressive anti-plasmodial assay and the effect on body weight and survival using the experimental *P. berghei* infected mice model. Whereas fixed oils and triterpenoids were the only detected phytochemicals in the LF, only amino acids, and carbohydrates were observed for HF with saponins, flavonoids, cyanogenic glycosides, anthraquinones and alkaloids absent in both fractions (see Table 3).

DISCUSSION

The result of the acute toxicity in Table 1 showed that both the lipophillic (LF) and hydrophilic (HF) fractions from the edible mushroom *P. ostreatus* are non-toxic as neither death nor visible signs of toxicity was observed even at the highest dose of 5000 mg/kgbw. This implies LD_{50} of greater than 5000 mg/kg bw. This is an indication of the high safety margin of the constituents in these two fractions from this edible mushroom. Four days suppressive anti-malarial test is a preliminary test for the determination of anti-malarial activity in *P. berghei* infected mice. In this study, both the lipophilic LF and hydrophilic HF fractions treated groups showed significant ($p < 0.05$) percentage parasitaemia suppression effect compared to the untreated group though not as active when compared to chloroquine (100 % suppresion). Although this effect was not significantly($p > 0.05$) dose dependent it could be regarded as active. A substance is considered to be active when the percentage suppression of parasitaemia is greater than 30 % [19-20]. Unlike the LF, mice treated the HF (1000 mg/kg bw) were observed to have a significant ($p , 0.05$) lesser weight reduction effect compared to the group treated with the standard drug chloroquine (see Table 2). This could be attributed to the adverse effect of chloroquine like anaemia, hypoglyceamia and loss of appetite. Thus the constituents in HF could help in the amelioration of these possible fatal side effects associated with malaria. The presence of amino acids and carbohydrates as constituents in the HF could offer a rationale for this trend. Fungal polysaccharides[3] and proteins[21] have been reported to exhibit immunomodulatory effects. Immunostimulatory water soluble polysaccharides like the Lentinans from the mushroom *Bulgaria inguinans* have been investigated for anti-malarial activity[3]. Generally,mice treated with the higher dose of the LF or HF, showed higher survival rate compared to lower dose . The presence of the lipophillic constituents: fixed oils and triterpenoids in the LF, and hydrophilic constituents:amino acids, and carbohydrates in the HF could offer a rationale for the observed trend in activity.

CONCLUSION

This observed trend in anti-plasmodial activity *in vivo,* corroborated the earlier reported *in vitro* activity[10] and is a further evidence supporting the nutraceutical potential in the management of malaria infection, of the edible mushroom *Pleurotus ostreatus* cultivated in Nigeria.. After further investigation, this edible mushroom species may be recommended in the diet as a prophylaxis against malaria infection.

ACKNOWLEDGEMENT

The authors gratefully acknowledged the Nigerian Institute for Medical Research (NIMR), Lagos, Nigeria for making available the *Plasmodium berghei* strain used for this study.

CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

REFERENCES

1) Malaria fact sheet N° 94. In: www.who.int/mediacentre/factsheets/fs094/en/ Accessed December 2015
2) Isaka, M, Tanticharoen, M, Kongsaeree, P, Thebtaranonth, Y. Structures of Cordypyridones A- D, Antimalarial *N*-Hydroxy- and *N*-Methoxy-2-pyridones from the Insect Pathogenic Fungus *Cordyceps*. *Journal of Organic Chemistry.* 2001; 66 (14) : 4803-4808
3) Bi, H, Han, H, Li, Z, Ni, W, Chen, Y, Zhu, J, Gao, T, Hao, M, Zhou, Y. A Water-Soluble Polysaccharide from the Fruit Bodies of *Bulgaria inquinans* (Fries) and Its Anti-Malarial Activity. *Evidence-Based Complementary and Alternative Medicine.* 2011; Article ID 973460: 1-12. doi.org/10.1093/ecam/neq070.
4) Meza-Menchaca, T, Suarez-Medellin, J, Angel-pina, CD; Trigos, A.The Amoebicidal Effect of Ergosterol Peroxide Isolated From *Pleurotus Ostreatus Phytotherapy Research.*2015*;* 29 (12): 1982-1986.

5) Ramos-Ligonio, A, Lopez-Monteon, A; Trigos, A. Trypanocidal Activity of Ergosterol Peroxide From *Pleurotus Ostreatus. Phytotherapy Research*. 2011; 26 (6): 938-943.

6) Degenkolb, T, Vilcinskas, A. Metabolites from nematophagous fungi and nematicidal natural products from fungi as alternatives for biological control part II: Metabolites from nematophagous basidiomycetes and non-nematophagous fungi. *Applied Microbiology and Biotechnology.*2016; 100: 3813-3824

7) Lull C, Wichers, HJ, Savelkoul, HFJ. Anti-inflammatory and Immunomodulating Properties of Fungal Metabolites. *Mediators of Inflammation;* 2005(2): 63–80. doi.org/10.1155/MI.2005.63.

8) Wu, JY, Chen, CH, Chang, WH, Chung, KT, Liu, YW, Lu, FJ, Chen, CH. Anti-cancer effects of protein extracts from *Calvatia lilacina*, *Pleurotous ostreatus* and *Volvariella volvacea. Evidence-based complementary and Alternative Medicine.*2011; *Article ID* 982368:1-10. doi.org/10.1093/ecam/neq057

9) Elsayed, AE, El Enshasy, H, Wadaan, MAM, Aziz, R."Mushrooms: A Potential Natural Source of Anti-Inflammatory Compounds for Medical Applications," *Mediators of Inflammation. 2014*; Article ID 805841:1- 15p. doi.org/10.1155/2014/805841.

10) Afieroho OE, Siwe Noundou X, Onyia CP, Festus OH, Chukwu EC, Adedokun OM, Isaacs M, Hoppe HC, Krause RWM, Abo KA. Antiplasmodial activity of the n-hexane extract from Pleurotus ostreatus (Jacq. Ex. Fr) P. Kumm. *Turkish Journal of Pharmaceutical Sciences; 2019; 16(1): Ahead* of Print:TJPS-18894

11) Afieroho, OE, Chukwu, *EC, Festus, OH, Onyia, CP, Suleiman, M, Adedokun, OM*. Evaluation of the anti-mitotic and bacteriostatic activities of the fruiting bodies of *Pleurotus ostreatus* (Jacq. Ex. Fr) P. Kumm. (Pleurotaceae). *Malaysian Journal of Medical and Biological Sciences.*2017; 4(1):15-20

12) Afieroho, OE, Lawson, L, Adedokun, OM, Emenyonu N. Antituberculosis and phytochemical investigation of the dichloromethane extract of *Pleurotus tuber-regium* (Fries) Singer sclerotium. *International Research Journal of Pharmacy.*2013; 4(1):255-257

13) Afieroho, OE, Ollornwi, KV, Elechi, N, Okwubie, L, Okoroafor, D, Abo, KA. Free radical scavenging potentials and level of some heavy metals in *Pleurotus flabellatus* Berk and Broome (Pleurotaceae) *The Global Journal of Pharmaceutical Research. 2013; 2 (3):1807-1812.*

14) Afieroho, OE, Ugoeze, KC. Gas Chromatography-Mass Spectroscopic (GC-MS) Analysis of n-Hexane Extract of *Lentinus tuber-regium* (Fr) Fr (Polyporaceae) Syn *Pleurotus tuber regium* Fr sclerotia. *Tropical Journal of Pharmaceutical Research.*2014; 13 (11): 1911-1915

15) Lorkes D. A new approach to practical acute toxicity testing. *Arch. Toxicol* 1983; 54(4): 275-287.

16) Peters W and Robinson BL. The chemotherapy of rodent malaria. XLVII. Studies on pyronaridine and other Mannich base antimalarials," *Annals of Tropical Medicine and Parasitology*, 1992: 86,(5):. 455–465, 1992.

17) Harborne, JB. Phytochemical Methods- a Guide to Modern Techniques of Plant Analysis 3rd ed. London: Chapman and Hall; 1998.

18) Houghton, PJ, Raman, A. (1999). Laboratory Handbook for the Fractionation of Natural Extracts. London: Chapman and Hall; . 1999

19) Krettli AU, Adebayo JO, Krettli LG. Testing of natural products and synthetic molecules. Aiming at new antimalarials. *Current Drug Targets*. 2009: 10(3):261-270.

20) Adugna M, Feyera T, Taddes W, Admasu P. *In vivo* anti-malarial activity of crude extract of aerial part of *Artemisia abbyssinica* against *Plasmodium berghei* in mice. *Global Journal of Pharmacology.* 2014: 8:460-8

21) Zhou LD, Zhang Y, Liu T, Cao Y.M . The shiitake mushroom derived immune stimulant lentinan protects against Murine malaria blood- stage infection by evoking adaptive immune response. *International Immunopharmacology* 2009: 9(4):455-462

Table 1: Result of acute toxicity evaluation of the lipophillic and polar fractions from *P.ostreatus* fruiting bodies against healthy mice model

Dose (mg/kg body weight)	Lipophillic fraction (LF)	Polar fraction (HF)
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
1600	0/3	0/3
2900	0/3	0/3
5000	0/3	0/3
LD_{50}	>5000 mg/kg bw	>5000 mg/kg bw

Table 2: Result of anti-plasmodial (four days chemosuppressive) activity of the lipophillic and polar fractions from *P.ostreatus* fruiting bodies against *P. berghei* infected mice model

Test sample	Dose per kg body weight	% Chemosuppression	% weight loss	% survival rate
Lipophillic fraction (LF)	1000 mg	70.91±0.72	26.10±3.05	40
	10 mg	71.67±1.07	20.60±0.77	20
DMSO	10 ml	0.00	28.80±2.51	20
Polar fraction (HF)	1000 mg	70.43± 8.33	3.95*±0.20	40
	10 mg	69.35±0.00	22.90±8.83	20
Distilled water	10 ml	0.00	27.62±6.17	20
Chloroquine	10 mg	100.00	25.50±1.18	100

*Significant ($p< 0.05$compared to the standard drug chloroquine

Table 3: Results of the phytochemical screening of the lipophillic and polar fractions from *P.ostreatus* fruiting bodies

Phytochemical constituents)	Lipophillic fraction (LF)	Hydrophilic fraction (HF)
Alkaloids	Absent	Absent
Saponins	Absent	Absent
Phenolics	Absent	Absent
Anthraquinones	Absent	Absent
Cyanogenic glycosides	Absent	Absent
Triterpenoids	present	Absent
Amino acids	Absent	present
Carbohydrates	Absent	Present
Fixed oils	Present	Absent

Lawsonia Inermis Leaf Extract Mitigates Aluminium-Induced Testicular Toxicity in Wistar Rats: Immunohistochemical Study

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ABSTRACT

Introduction

Apoptosis is a genetically controlled cell suicide pathway which plays an essential role in deleting excess, unwanted or damaged cells during development and tissue homeostasis. Phytochemical screening of the *Lawsonia inermis* leaf extracts had showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids.The aim of this study was to investigate the mitigating effect of *Lawsonia inermis* aqueous leaf-extract on aluminium-induced oxidative stress on the testes of adult Wistar rats' as determined by immunohistochemistry.

Materials and Methods

Thirty five adult male Wistar rats, weighing between 100-196g. *Lawsonia inermis* extracts and aluminum chloride ($AlCl_3$) were dissolved in distilled water then administered for a period of three (3) weeks through the oral route with Five (5) rats per group. Group 1 (control): Given rat pellets and distilled water. Group 2: Given 60mg/kg/d extract of aqueous *Lawsonia inermis*. Group 3: Given 0.5mg/kg/d of $AlCl_3$. Group 4: Given 0.5mg/kg/d of $AlCl_3$ and 60mg/kg/d of aqueous *Lawsonia inermis* orally. Group 5: Given 0.5mg/kg/d of $AlCl_3$ and 75mg/kg/d of aqueous *Lawsonia inermis* orally. Group 6: Given 0.5mg/kg/d of $AlCl_3$ and 100mg/kg/d of aqueous *Lawsonia inermis* orally. Group 7: Given 0.5mg/kg/d of $AlCl_3$ and 5mg/Kg/d Ascorbic acid in distilled water orally. Twenty four hours after the last administration, the animals were weighed, anaesthesised with chloroform pituitary glands were located, removed and weighed using an electronic sensitive analytical balance.

Results

The densities of Bax, ki 67 and Bcl2 were highest in the control rat followed by the rats given 60mg/kg extract of *Lawsonia inermis* while the rats given 0.5mg/kg/d of aluminum chloride were the lowest.

Conclusion

The immunoreactivities of other rats increased according to the dosage. In conclusion, aluminum chloride decreased the immunoreactivities germ cells thus normal apoptosis was altered but *Lawsonia inermis* ameliorated/ mitigated this deleterious effect of aluminum chloride.

Key Words: Apoptosis, Autophagy. Carcinogenesis, Entosis

INTRODUCTION

Multicellular organisms have several cells, and in other to maintain the homeostasis in the body of many of these organisms many of their cells die every day. We have different types of cells death: necrosis, apoptosis, autophagy and entosis. Necrosis is due to external factors such as toxins, infection, and/or trauma which elicits immune response while apoptosis is known as-programmed cell death which does not cause lysis of the cell, nor inflammatory response[1]. Autophagy is degradation of own cells by lysosomal reaction, while entosis involves cell destroying neighboring cell [2, 3].

Apoptosis is a physiological process which helps in maintaining homeostasis drilling the body by replacing old cells with new cells. It is said to be a genetically controlled cell suicide pathway.

Pathological dysfunction of the apoptotic pathway may result in carcinogenesis[4]. Some tumor cells interfere with the process of apoptosis leading to immortality. One of the genes that are implicated is: Bcl-2. Over expression of Bcl2 causes carcinogenesis[5,6].

Carcinogenesis is induced when the programme cell death is impaired[7]. Bcl2 expression is high with tumors less than 550g and with favourable condition[8-10]. Bcl2 is an anti-apoptotic protein which inhibits apoptotic death while Bax protein is pro apoptosis which enhances the apoptotic death[11]. Ratio of Bcl2/Bax determines the induction as well as inhibition of apoptosis in human. When the ratio is greater than >1anti apoptotic signal is dominant and when the ratio is <1 apoptotic mechanism dominates. Apoptosis occurs in vertebrate body and there is high incidence in male organ (testis) about 75% of all the germ cells produce are renewed through apoptosis[12].

The spermatogenic process occurs within the seminiferous tubule of the testis and it involves spermatogenesis maturation into spermatozoa. The cells production of spermatocytes within seminiferous tubules is dependent on the dynamic balance between programmed cell death and cell proliferation[13], as with other cells of the body. During embroyological development, excessive cells generated due to apoptosis[14], or over expression of Bax is often observed during the early stages of germ cell development of the testes[15].

Ki-67 expression is universally acceptable as proxy for cell proliferation activity and Ki-67 labelling index correlates with the growth of many human neoplasms[16]. Ki-67 is prominent during the cell cycle but not in GO phase. Ki-67 is located on chromosome 10. Both the sexual hormones and male germ cell apoptosis regulate germ cell survival; however, excess or low hormones can induce cellular apoptosis in the testis[17].

Sertoli cells can serve as hormone regulators of spermatogenesis they have receptors for FSH and testosterone. Cessation of hormone secretion can lead to germ cell apoptosis[18]. Both FSH, testosterone and oestradiol assists during germ cell maturation. The homeostasis of germ cells is maintained by FSH and testosterone level[19, 20]. It has been reported that, excessive release of testosterone could result in death while testosterone deprivation elicits caspace activity which could induce DNA fragmentation in sertoli cells[21].

The potential of herbal medicines and medicinal plant in health care delivery, particularly in the third world is no longer in doubt having gained recognition in several nations of the world and by the World Health Organization (WHO) [22-24]. *Lawsonia inermis* commonly known as henna belongs to the family Lythraceae and genus Lythrum. Henna is a common name for a small shrub and for the dye that is obtained from its leaves. The shrub is also called alkanna mignonette tree, El-henna and Egyptian priest. Phytochemical screening of the henna leaf extracts has shown the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids[24,25].

According to Jain[26], the phytochemical investigation of henna leaf shows total ash (14.60%), acid insoluble ash (4.50%) and water soluble ash (3.0%). Alcoholic extract and aqueous extract carbohydrate, glycosides, tannins, phenolic compounds, gums and mucilage were present in good quantity but saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, volatile oils were absent. There is evidence of the plant having wound healing properties[27].

Aluminum is known to be the most abundant metal and the third most common element in the earth's crust2[8, 29]. It is found abundantly as trioxosilicate (IV) in rocks and Clays. Chemically, it is often found in combination with silicon, fluorine, oxygen, and other earth elements[30]. It was reported that the oral bioavailability of aluminum can be as low as 0.1%; after absorption, it is distributed into the body of animal and human tissues and bones[31-32]. Aluminum ion is transported in the plasma by the iron binding protein, transferrin and it can enter the brain, placental and fetus[33, 34]. Aluminum has a longer half life in humans than in rodents.

Aluminium has been reported to have no biological function[35] but it has some important functions in most

of the developing countries. Aluminum can be used in making utensils, cookware, cosmetics, cointainers, and aluminum foil; other primary sources of aluminum includesalt, yellow cheese, corn, herbs, teas and spices[36-39]. Medically, aluminum has been used in the production of antacids, vaccines and injectable allergens, as phosphate binders and in buffered aspirin[40-42]. It is also used as an additive in toothpaste and food[43]. Aluminum can also be found naturally or included in drinking water and food products[44]; In fact it is believed to not be toxic but it has adverse effects on human health[45].

MATERIALS AND METHODS

Collection and Preparation of Extracts

The plant was obtained from Isanlu-Isin in Kwara State, Nigeria and identified professionally with the Herbarium number **UPH/P/114** by the Taxonomist in the Department of Plant Science and Biotechnology, University of Port-Harcourt, Rivers State, Nigeria. The Research ethics Committee of the same institution approved this work on 25th February, 2016 with reference number **UPH/CEREMAD/REC/04.** The plant leaves were washed with water, cut into pieces, dried in a cool environment. The dried plant leaves were pulverized into coarse powder in a grinding machine. The filtrate was concentrated using Rotary evaporator (Buchi) and further concentrated to dryness at 50°c in an electric oven (GallenKamp).After drying it was stored in the refrigerator at 4°c until needed for use.

Drugs and Chemicals

Aluminium Chloride and Ascorbic acid were bought from Mich-Deson Hospital Equipment store, Upper Taiwo, Ilorin. The histological staining was done in the Anatomical-pathology Department of the University Teaching Hospital, Ilorin and immunohistochemical staining was done in University College Hospital, Ibadan.

Acute Toxicity Test (LD_{50}): Fifteen mice were used to conduct the above test to determine the safe dosages and lethal dosage. They were grouped into five (5), with three (3) mice per group. The acute toxicity of the Aqueous Extract of *Lawsonia inermis* extract was assessed by LD_{50} calculation, using a limit dose test at a limit dose of 1000mg/kg body weight of the extract after oral administration in mice (three animals per group) (OECD-OCDE 425 Guide).

Determination of the Dosage of the Extract to Administer: The choice of dosage based on the acute toxicity test (LD_{50}) above, the safe dose of *Lawsonia inermis*is 0.1g/Kg or 100mg/Kg body weight. The highest dose is 100mg/Kg, medium dose is 75mg/Kg and the lowest dose is 60mg/Kg.

Breeding of the Animals: Thirty five adult male Wistar rats and fifteen mice were used, with an average weight of 100-196g. The rats, after procurement, were housed in cages (made from wood, wire gauze and net) under natural light and dark cycles at room temperature in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. The floor of the cages were made with wood to make it comfortable for the rats and it was covered with sawdust to provide a soft floor for the rats and to make cleaning of the cage convenient when littered. They were fed with rat pellets purchased from approved stores by University of Ilorin, and water was given *ad libitum.* They were grouped and left to acclimatize for 2 weeks before the study commences.

Grouping: The total numbers of animals were thirty five. They were grouped into one (1) control and six (6) experimental groups with consideration towards size variation. Using a feeding tube (size-6), distilled water, and *Lawsonia inermis* extracts were administered to the control and treated animals respectively for a period of three (3) weeks.

Group 1 (control): (n = 5): Given rat pellets and distilled water.

Group 2: (n = 5): Given 60mg/kg/d extract of *Lawsonia inermis* and pellets.

Group 3: (n = 5): Given0.5mg/kg/d of aluminum chloride in distilled water and pellets.

Group 4: (n = 5): Given 0.5mg/kg/d of aluminum chloride and low dose 60mg/kg/d of *Lawsonia inermis* in distilled water orally.

Group 5: (n = 5): Given 0.5mg/kg/d of aluminum chloride and medium dose 75mg/kg/d of *Lawsonia inermis* orally.

Group 6: (n = 5): Given 0.5mg/kg/d of aluminum chloride and high dose 100mg/kg/d of *Lawsonia inermis* in distilled water orally.

Group 7: (n=5): Given 0.5mg/k/d of aluminum chloride and 5mg/Kg/d Ascorbic acid in distilled water orally.

Animal Sacrifice and Sample collection: Twenty four hours after the last administration, the animal were weighed and thereafter sacrificed by the use of chloroform as an anesthetic. Abdominal cavity was opened by a midline abdominal incision and the reproductive organs (Testes) were removed.

Immunohistochemical Staining Method

The two testes were embedded in paraffin blocks.These tissues were sectioned serially, at least two non-serial sections were stained with hematoxylin and eosin (H&E). The Leydig cells were identified by staining for 3b-HSD. The 5mm sections were mounted, then dewaxed and rehydrated.

In other to perform, antigen retrieval was performed by pressure cooking slides in 0.01M citrate buffer (pH 6.0) about 5minutes and then incubated in 3% (vol/vol) of H_2O_2 in methanol to block endogenous peroxidase activities for 30 minutes, then slides were washed in Tris-buffered saline [TBS; 0.05M Tris, 0.85% (wt/vol) NaCl (pH 7.4)]. No specific binding sites were blocked with an appropriate normal serum diluted at 1:5 in TBS containing 5% (wt/vol) BSA.

For proper incubation of tissue overnight, addition of goat polyclonal antibody against 3b-HSD incubation at 4 _C and 3b-HSD antibody was recommended for detection of 3b-HSD1 or 3b-HSD2 in mouse tissues. It was then wash in Tris-buffered saline (TBS).

Moreso, the incubation of slide was for 30 minutes;using the suitable secondary antibody conjugated to biotin rabbit anti-goat (Santa Cruz Biotechnologies). Dilution into 1:500 in the blocking mixture. Incubated with horseradish peroxidase-labeled avidin-biotin complex for 30minutes, followed by application of diaminobenzidine for immunostain develop.

Lastly, counterstained with hematoxylin, dehydrated then mounted, using Pertex mounting medium. The number of 3b-HSD-positive cells was counted in 12 randomly selected fields from each slide at a magnification of x400.

Immunohistochemical detection of BCL-2

Immunohistochemical detection of BCL-2 was done using avidin-biotin-peroxidase complex with monoclonal antibodies against BCL-2. The section was cooled at room temperature for 20 minutes, followed by retrieval and then incubated for 30 minutes with 10% normal horse serum.

Excess serum were decanted away, the sections were then incubated with primary antibody for 20 hours at room temperarure; After that, DO7 monoclonal antibody was used at a 1:100 dilution. The sections were subsequently incubated with pre-diluted biotinylated anti-mouse immunoglobulin for 30 minutes at 37°C.

The sections were washed with PBS, and allowed to react with peroxidise-conjugated at dilution of 1:500 for 30 minutes at 37°C. Lastly, PBS, peroxidise activity was evaluated with 3,3'-diaminobenzidine tetrahydrochloride (DAB), and the sections were counterstained with Harris hematoxylin.

Immunohistochemical detection of BAX

The avidin-biotin-peroxidase complex was employed to achieve immunohistochemical procedure. Following antigen retrieval and cooling for 20minutes, incubation of section was done with 10% normal horse serum (Vectastain Elite kit) for 30minutes. After decanting away the excess serum, sections were incubated with primary antibody for 2hours at 37°C. The monoclonal antibody BAX (policlonal, Dako®, 1:50), was used at 1:500 dilution (Pharmingen, USA). The sections were subsequently incubated with pre-diluted biotinylated anti-mouse immunoglobulin (Vectastain Elite kit) for 30 minutes at 37°C. After washing with PBS, the sections were reacted with peroxidase-conjugated streptoavidin (Dako, Denmak) diluted at 1:500 for 30 minutes at 37°C. After washing with PBS, peroxidase activity was evaluated with 3, 3'-diaminobenzidine tetrahydrochloride (DAB), and the sections were counterstained with Harris hematoxylin.

RESULTS

Plate A: Bax, Immunoreactivities micrograph for different groups. The arrows show some cells in the seminiferous tubules which are immunopositive, most are elongated spermatids, spermatozoa and few spermatocytes, spermatogonia. Magnification x400.

Plate B: BCL-2 Immunoreactivities micrograph for different groups. The arrows show some cells in the seminiferous tubules which are immunopositive, most are elongated spermatids, spermatozoa and few spermatocytes, spermatogonia. Magnification x400.

Plate C: Ki-67 Immunoreactivities micrograph for

different groups.. The red arrows show some cells in the seminiferous tubules which are immunopositive, most are elongated spermatids, spermatozoa and spermatogonia. Magnification x400.

Figure 1.0: Densities of Bax, Bcl-2 and Ki-67 Immunopsitive cells per tubules
Table 1.0: Densities of Bax, Bcl-2 and Ki-67 Immunopositive cells per tubules for the wistar rats
Immunoreactivities of cells were demonstrated in the table and figure 1.0 above, the densities of Bax, ki 67 and Bcl2 were highest in the control rat followed by the rats given 60mg/kg extract of *Lawsonia inermis* while the rats given 0.5mg of aluminum chloride per kg of body weight were the lowest. The immunoreactivities of other rats increased according to the dosage.

DISCUSSION

In this current study by the oral route, the animals showed dose-dependent signs of toxicity, ranging from lack of appetite, depression, immobility and respiratory distress to death. LD_{50} for *Lawsonia inermis* extract is 0.75g while the safe dose is 0.1g/Kg b.w.

The immunoreactivities of the cells were demonstrated in the table and figure 1.0 above the densities of both Bax, ki 67 and Bcl2 were highest in the control rat followed by the rats given 60mg/kg extract of *Lawsonia inermis* while the rats given 0.5mg of aluminum chloride per kg of body weight were the lowest. The immunoreactivities of other rats increased according to the dosage, that is: immunoreactivities of the rats that received low dose of *Lawsonia inermis* aqueous leaf-extract and aluminum chloride were lower compared to the highest dose with aluminum chloride .This implies, *Lawsonia inermis* aqueous leaf-extract ameliorates/ mitigate the oxidative stress induced by aluminum chloride toxicities.

The immunoreactivities of the cells demonstrated in the table and figure 1.0 above the densities of Bax, ki 67 and Bcl2, shows that the rats given 60mg/kg extract of *Lawsonia inermis* were equally having high density. According to Inder [46], *Lawsonia inermis* contains many dietary elements including both micro and micronutrients (carbohydrate, glycosides, tannins, phenolic compounds, gums, mucilage, saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, D-Glucopyranoside, methyl (51.73%) and 1, 4-Naphthalenedione, 2-hydroxy- 'Synonyms: Henna glycosides', phytosterol, steroids, saponins, tannins and flavonoids). This suggests its ability to influence many physiological processes. Many of the elements found in *Lawsonia inermis* have been demonstrated to have positive roles in spermatogenesis and testicular steroidogenesis.

The immunoreactivities of the rats increased according to the dosage, it was obvious in our study that the rats given low dose of *Lawsonia inermis* aqueous extract and aluminum chloride were lower compared to the rats given medium, highest dose of aluminum chloride. According to Shaha[17] reported that sexual hormones and programme cell death regulate germ cells. Shortage and over-production of hormones can cause cellular programme cell death in the testis. Alteration of hormone can lead to apoptosis[18]. During seminiferous tubule maturation, testosterone alongside the synergistic action of FSH and oestradiol assist in ensuring germ cell survival while oestradiol on its own acts as an inhibitor, with a pro-apoptotic effect. Ruwanpura reported sudden alteration of FSH could lead to spermatogonia[47].

Excess testosterone can cause as reported by Zhou[20]. In contrast, withdrawal of testotesrone stimulates the activity caspase which cause fragmentation of DNA in Sertoli cells and weak effect in germ cells [21]. Ki-67 expression is widely accepted as a measure of cell proliferative activity and the Ki-67 labeling index correlates with the growth of many human neoplasms[16]. Ki-67 is expressed during the G1, S, G2 and M phase of continuously cycling cells, but is absent in G0 cells. Ki-67 expression is more prominent during cell cycle of sperm cells. Rodrigue reported that toxicant influences survival rate of the germ cells and these induce programme cell death[48]. These could be as a result of toxicant or oxidative stress induced by aluminum chloride but table 1; plates A, B and C, show that addition of different doses of *Lawsonia inermis* ameliorated deleterious effect of aluminum chloride.

The immunoreactivities of the cells demonstrated in the table and figure 1; also plates A, B & C, above shows that the densities of Bax, ki 67 and Bcl2 were very low in the rats given 0.5mg of aluminum chloride per kg of body weight. Oxidative stress induced by aluminum chloride may disrupt molecular machinery

that control germ cell cycle especially at the *stage* of morphological differentiation producing failure of mature spermatids to disengage from their relation with Sertoli cells. It is not entirely clear if the mammalian testis is equipped with the ability to mount an active fight back to ongoing testicular insult. Endocrine disruptors affect hormones regulation and alters normal apoptosis according to McGlyn[49]. Usually, when there is hormonal disturbance, sperm production goes into recession and future recovery is dependent on the nature, the degree, and the target of the insult[50, 51].

CONCLUSION

The effect of *Lawsonia inermis* aqueous leaf-extract on spermatogenesis is registered molecularly and this was indicated by increase in *BCL2, Bax and Ki-67 densities* while the negative effects of aluminum toxicity activate *BCL2, Bax and Ki-67* -dependent pathway(s) which often result in apoptosis of cells from lethally damaged cells. Aluminum chloride decreased the immunoreactivities germ cells thus normal apoptosis was altered but *Lawsonia inermis* ameliorated/ mitigated this deleterious effect of aluminum chloride.

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REFERENCES

1. Kara H, Karatas F, Canatan H and Servi K. Effects of exogenous metallothionein on acute cadmium toxicity in rats. *Biology of Trace. Element Research. 2005;104(3)*:223-232.
2. Stromhaug PE and Klionsky D J. Approaching the molecular mechanism of autophagy. Traffic. 2001;2: 524–531.
3. Overholtzer M, Mailleux AA, Mouneimne G, Normand G, Schnitt SJ, King RW, Cibas ES and Brugge JS. A non apoptotic cell death process, entosis, that occurs by cell-in-cell invasion. Cell 2007;131: 966–979.
4. Osford SM, Dallman CL, Johnson PW, Ganesan A, Packham G. Current strategies to target the anti-apoptotic bcl-2 protein in cancer cells. Current Medical and Chemical Apr. 2004;*11(8)*: 1031-1039.
5. Kumar R, Vadlamudi RK, Adam L. Apoptosis in mammary gland and cancer. Endocrine-Related Cancer. 2000;*7*: 257-269.
6. Krajewski S, Krajewska M, Turner BC, Pratt C, Howard B, Zapata JM, Frenkel V, Robertson S, Ionov Y, Yamamoto H, Peruch M, Takayama S, Reed JC. Prognostic significance of apoptosis regulators in breast cancer. Endocrine-Related Cancer. 1999;*6*: 29-40.
7. Ghanem M A, der Kwast T HV, Hollander JDC, Sudaryo MK, den Heuvel MMV, Noordzij MA, Nijman RJM, Soliman HE, Steenbrugge GJV. The prognostic significance of apoptosis-associatedproteins BCL-2, BAX and BCL-X in clinical nephroblastoma. *British Journal of Cancer. 2001;*85 (10) 1557-63.
8. Antonsson B, Martinou JC. The Bcl-2 protein family. *Experimental Cell Research*.2000;256 (1) 50-57.
9. Re GG, Hazen-Martin DJ, El Bahtimi R, Brownlee NA, Willingham MC, Garvin AJ. Prognostic significance of Bcl-2 in Wilms' tumor and oncogenic potential of Bcl-X(L) in rare tumor cases. International Journal of Cancer. 1999;84(2):192–200.
10. Weeks DA, Beckwith JB, Luckey DW. Relapse-associated variables in stage I favorable histology Wilms' tumor. A repot of the National Wilms' tumor Study. *Cancer*. 1987;60 (6) 1204-12.
11. Sakuragi N, Salah A, Watari H, Itoh T, Inoue S, Moriuchi T, Fujimoto S. Bax, bcl-2 and p53 expression in endometrial cancer. Gyn Oncol. 2002;*86(3)*: 288-296.
12. Hoeppner DJ, Hengartner MO and Schnabel R. Engulfment genes cooperate with ced-3 to promote cell death in Caenorhabditis elegans. Nature. 2001;412: 202–206.
13. Russell LD, Chiarini-Garcia H, Korsmeyer SJ, Knudson CM. Bax-dependent spermatogonia apoptosis is required for testicular development and spermatogenesis**.** 2002.
14. Rucker III EB, Dierisseau P, Wagner KU, Garrett L, Wynshaw-Boris A, Flaws JA and Hennighausen L. Bcl-x and Bax regulate mouse primordial germ cell survival and apoptosis during embryogenesis. Molecular Endocrinology. 2000;14, 1038–1052.
15. Rodriguez I, Ody C, Araki K, Garcia I and

Vassalli P. An early and massive wave of germinal cell apoptosis is required for the development of functional spermatogenesis. Embryology Journal. 1997;16, 2262–2270.
16. Fontana D, Bellina M, Gubetta L. Monoclonal antibody Ki-67 in the study of the proliferative activity of bladder carcinoma. Journal of Urology. 1992;48: 119-1151.
17. Shaha C. Germ cell apoptosis: relevance to infertility and contraception. Immunology, Endocrinology, Metabolic Agents, Medicine and Chemistry. 2008;8: 66–78.
18. Sofikitis N, Giotitsas N, Tsounapi P, Baltogiannis D, Giannakis D and Pardalidis N. Hormonal regulation of spermatogenesis and spermiogenesis. Journal of Steroid Biochemistry,. Molecular Biology. 2008;109, 323–330.
19. Pareek TK, Joshi AR, Sanyal A, Dighe RR. Insights into male germ cell apoptosis due to depletion of gonadotrophins caused by GnRH antagonists. Apoptosis. 2007;12: 1085–1100.
20. Zhou XC, Wei P, Hu ZY, Gao F, Zhou RJ and Liu YX. Role of Fas/FasL genes in azoospermia or oligozoospermia induced by testosterone undecanoate in rhesus monkey. Acta Pharmacologica Sinica. 2001;22: 1028–1033.
21. Tesarik J, Martinez F, Rienzi L, Iacobelli M, Ubaldi F, Mendoza C, Greco E. In-vitro effects of FSH and testosterone withdrawal on caspase activation and DNA fragmentation in different cell types of human seminiferous epithelium. Human Reproduction. 2002;17: 1811–1819.
22. Paul CW, and Ligha AE. The Relationship between hydro-methanol leaf extract of lemon grass (Cymbopogon Citratus) and the histology of Albino Wistar Rats' Pancreas; Journals of Anatomical Sciences. 2015;6(1): 30-38.
23. World Health Organization. Traditional medicine Media centre; (Revised Dec 2008; Cited 2 Dec 2009). http:// www..who.int/media-centre/facts sheet/fs34/cn/. 2008.
24. Olawuyi TS, Olorunfemi OJ and Aina OS. Effect of *Lawsonia inermis* Leave extract in Aluminium- induced oxidative stress on Biochemical Parameters of Adult Male Wistar rat. European Journal of Biomedical and Pharmaceutical Sciences, 2017; 4(4): 62-69.
25. Raja W, Ovais M, Dubey A. Phytochemical Screening and Antibacterial Activity of *Lawsonia inermis* Leaf Extract. International Journal of Microbiological Research. 2013;4.
26. Jain GC, Pareek H, Sharma S, Bhardwaj B and Khajja BS. Reproductive toxicity of vanadyle sulphate in male rats. Journal of Health Sciences. 2007;53: 137-141.
27. Jain VC, Shah DP, Sonani NG, Dhakara S, Patel NM, Ellis RP. Pharmacognostical and preliminary Phytochemical investigation of Lawsonia inermis L. Leaf. Rom Journal of Biology. 2010;55(2): 127-133.
28. Ali BH, Basir AK, Tanira MOM. Anti-inflamatory, anti-pyretic and analgesic effect of *Lawsonia innermis, L* (Henna) in rats Pharmacology. 1995;51: 356-363.
29. Mohammadirad A and Mohammad A. A systematic review on oxidant/ Antioxidant imbalance in Aluminium toxicity. International journal of Pharmacology. 2011;7 (1): 12-21.
30. Onyegeme-Okerenta BM, Anacletus FC. Evaluation of aluminium toxicity and the ameliorative effect of some selected antioxidants on fecundity of matured male wister rats (rattus rattus). *European journal of advanced research in biological and life sciences.* 2016;4 (2): *2056-5984.*
31. Sigel H and Sigel AE. Metal ions in dialysis demenia syndrome and aluminum intoxication. Nephron. 1988;31:1-10.
32. Aguilar-Fuentes J, Fregoso M, Herrera M, Reynaud E, Braun C, Egly JM, Zurita M. p8/TTDA overexpression enhances UV-irradiation resistance and suppresses TFIIH mutations in a Drosophila trichothiodystrophy model. PLoS Genetics. 4(11): e1000253.
33. Walker JA, Sharman RA, Cody R.P. The effect of oral bases on enteral aluminium absorption. Arch International Medicine. 1990;150 (10): 2037-2039.
34. Macedo MF, De-Sousa M. "Transferrin and the transferrin receptor: of magic bullets and other concerns". Inflammation & Allergy Drug Targets. 2008;**7** (1): 41–52.
35. Fleming J, and Josh JG. Ferritin: Isolation of aluminium-ferrittin complex from brain. Proc.

Academic Sciences. 1987;84: 7866-7870.
36. Lin JL, Yang YJ, Yang SS, and Leu ML. Aluminum utensile contribute to aluminum accumulation in patients with renal disease. American Journal of Kidney Disease. 1997;30: 653-658.
37. Becaria A, Campbell A, and Bondy SC. Aluminium as a toxicant. Toxicology and Industrial Health. 2002;18: 309-320.
38. Sharma P and Mishra K. Amelioration of fumonisin B1 hepatotoxicity in mice by depletion of T cells with anti-Thy-1.2. Reproduction Toxicology. 2006;21: 313-321. http://www.ncbi.nlm.nih.gov/pubmed/16690192
39. Pournourmohammadi S, Khazadi P, Eslamizad A, Tajvar A, Mohammadirad and Abdollahi M. Study on the oxidative stress status among cement plant workers; Human Experimental Toxicology. 2008;27: 463-469.
40. Cannata J, Briggs J, Junor R, snd Fell S. Aluminum hydroxide intake real risk of aluminum toxicity. Br. Med. J. 1983;286: 1937-1938. .
41. Kaehny WD, Hegg AP and Alfrey A. Gastrointestinal absorption of aluminum from aluminum containing antacids. New England Journal of Medicine. 1977;296: 1389-1390. http://www.ncbi.nlm.nih.gov/pubmed/859546
42. Lione A. Aluminum toxicity and the aluminum containing medication. Journal Pharmacology Ther. 1985;29: 255-285.
43. Abbasali KM, Zhila T, and Farshad N. Developmental toxicity of aluminum from high doses of AlCl3 in mice. Journal of Applied Research. 2005;5: 575-579.
44. Yokel RA and McNamara PJ. Aluminum toxicokinetics: An updated mini review. Pharmacol. Toxicol. 2001;88: 159-167.
45. Osinska E, Kanoniuk D and Kusiak A. Aluminum hemotoxicity mechanisms. Ann. University Mariae Curie Sklodowska. 2004;59: 411-416.
46. Inder, K. M. Lawsonia inermis - From traditional use to scientific assessment; Manipal- 2011;576, 104.
47. Ruwanpura SM, McLachlan RI, Stanton PG and Meachem SJ. Follicle-stimulating hormone affects spermatogonial survival by regulating the intrinsic apoptotic pathway in adult rats. Biology of Reproduction. 2008a;78: 705–713.
48. Rodriguez JM, Glozak MA, Ma Y, Cress WD. Bok, Bcl-2-related ovarian killer, is cell cycle-regulated and sensitizes to stress-induced apoptosis. Journal of Biological Chemistry. 2006;281: 22729–22735.
49. McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV and Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. Journal of Nature and Cancer Institution. 2008;100, 663–671.
50. McLachlan RI, Wreford NG, Tsonis C, De-Kretser DM and Robertson DM. Testosterone effects on spermatogenesis in the gonadotrophinreleasing hormone-immunized rat. Biology of Reproduction. 1994;50 271–280.
51. Walker WH and Cheng J. Follicle stimulating hormone and testosterone signaling in sertoli cells. Reproduction. 2005;*130: 15-28.*

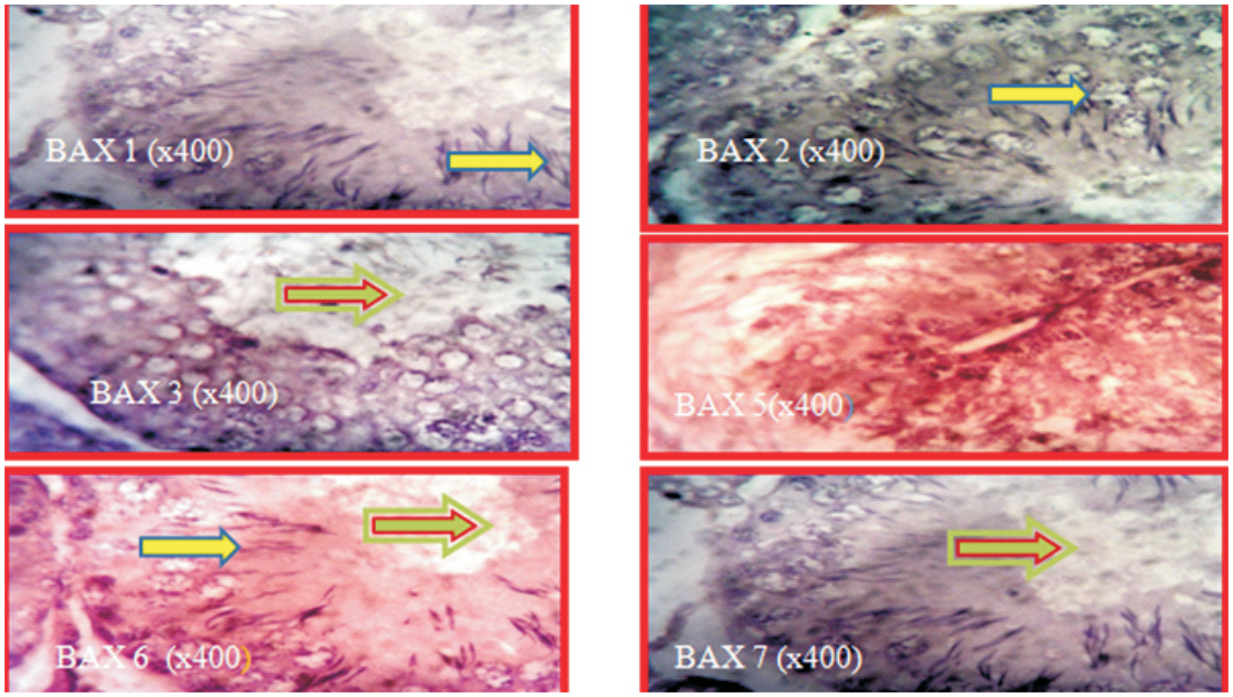

Plate A: Bax, Immunoreactivities micrograph for different groups. The arrows show some cells in the seminiferous tubules which are immunopositive, most are elongated spermatids, spermatozoa and few spermatocytes, spermatogonia. Magnification x400.

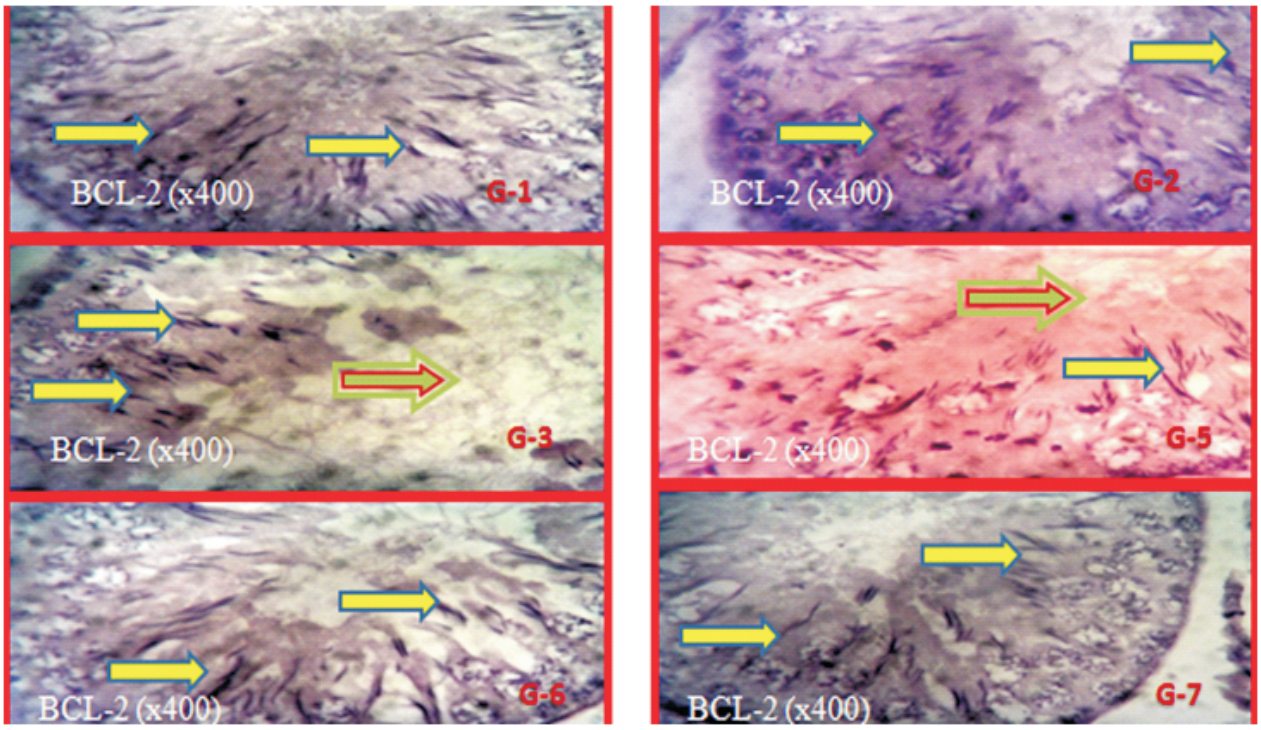

Plate B: BCL-2 Immunoreactivities micrograph for different groups. The arrows show some cells in the seminiferous tubules which are immunopositive, most are elongated spermatids, spermatozoa and few spermatocytes, spermatogonia. Magnification x400.

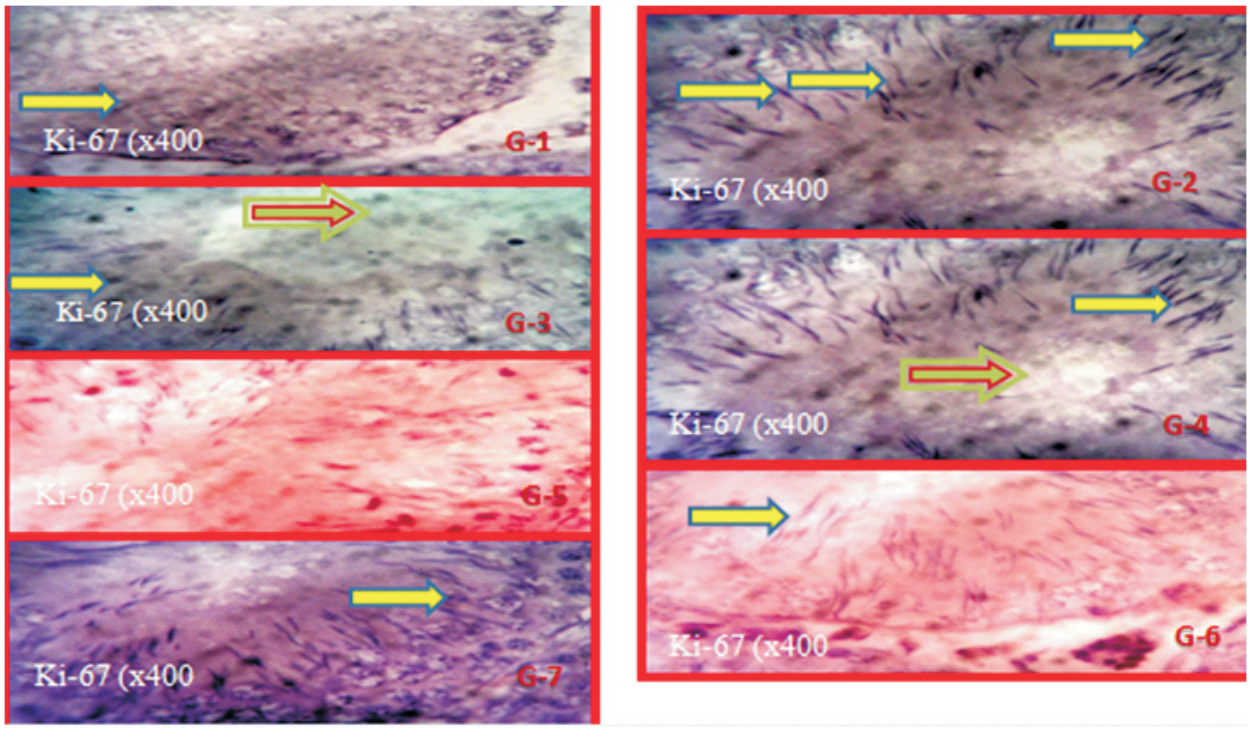

Plate C: Ki-67 Immunoreactivities micrograph for different groups.. The red arrows show some cells in the seminiferous tubules which are immunopositive, most are elongated spermatids, spermatozoa and spermatogonia. Magnification x400.

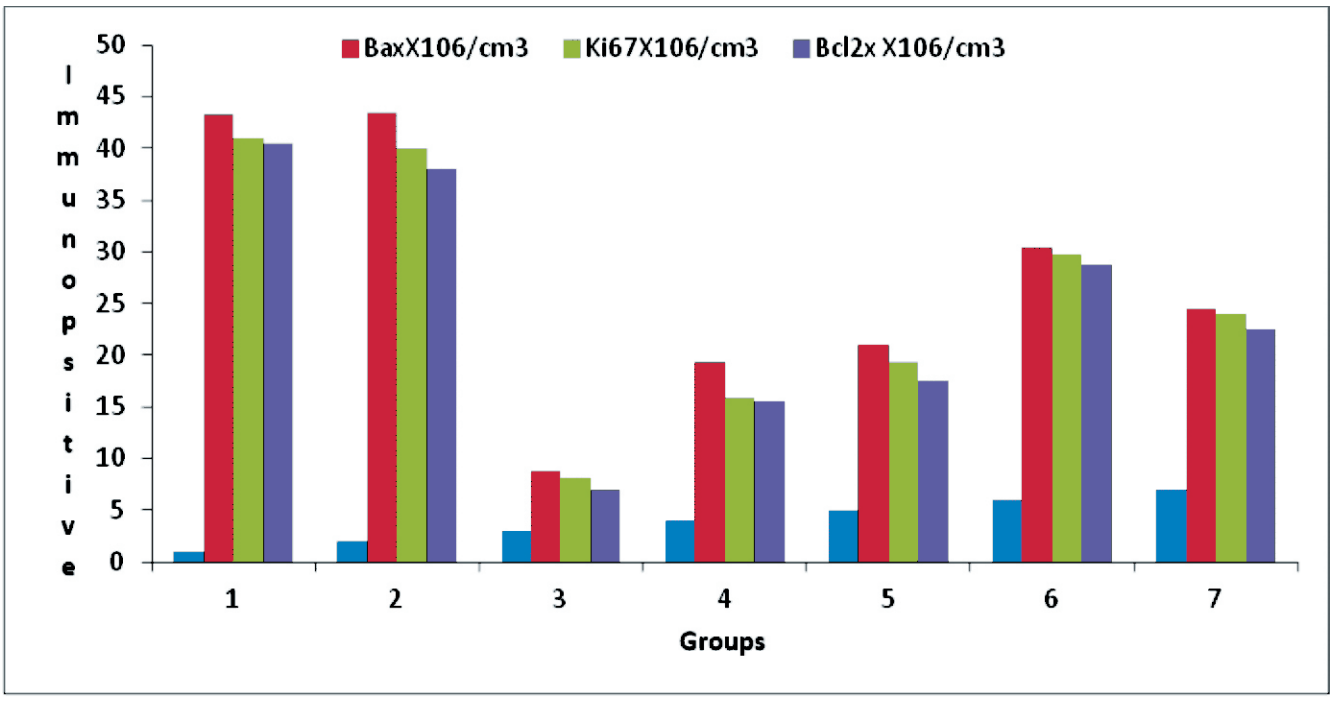

Figure 1.0: Densities of Bax, Bcl-2 and Ki-67 Immunopsitive cells per tubules

Table 1.0: Densities of Bax, Bcl-2 and Ki-67 Immunopositive cells per tubules for the wistar rats

Group	BaxX106/cm3	Ki67X106/cm3	Bcl2x X106/cm3
GRP 1	43.2± 2.2	41± 2.1	40.5± 1.9
GRP 2	43.4± 2.3	40± 1.4	38± 1.1
GRP 3	8.8± 2.1	8.1± 0.6	7± 0.4
GRP 4	19.3±1.1	15.8±0.9	15.6±0.9
GRP 5	21±1.3	19.3± 1.3	17.5±1.0
GRP 6	30.4±2,0	29.8±1.9	28.7±1.8
GRP 7	24.5±1.4	23.9±1.4	22.5±1.1

Interleukin 8 and Transforming Growth Factor-Beta in Patients With Malaria in Lagos State, Nigeria

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ABSTRACT

Introduction

The interaction between pro- and anti-inflammatory cytokines such as interleukin-8 (IL-8) and transforming growth factor beta (TGF-β) plays an important role in malaria pathogenesis and outcome. TGFβ, produced by a wide range of cells, has a pivotal role in the control of the transition between pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type) response during the acute and resolving phases of malaria infection. The role of IL-8 in *P. falciparum* malaria is unknown although studies indicate its likely use as a biomarker of intensity of malaria. The aim of this study was to measure the plasma levels of IL-8 and TGF- βin 136 individuals with malaria and correlate the production of these cytokines with the severity of the disease

Method

IL-8 and TGF- βlevels were determined using enzyme-linked immunosorbent assay. The severity of malaria was established by parasitemia, clinical symptoms and haematological parameters.

Results

Patients with malaria had a mean IL-8 level of 508.8±755.1pg/ml compared to controls who had no detectable levels of plasma IL-8.The difference in the mean between both groups was statistically significant ($p<0.05$). The level of parasitemia among patients with high IL-8 concentration and low IL-8 concentrations given as 21,097±24,340.4parasites/µL and 43,200±75,819.1parasites/µL, respectively, was observed and the difference in the mean levels of parasitemia between high and low chemokine levels was statistically significant ($p<0.05$). Amongst patients with low levels of TGF-β mean packed cell volume were within normal average ranges (34.55±8.0%) whereas patients with high TGF-β levels had mean packed cell volume below normal average ranges (29.20±12.5%). This difference in mean packed cell volumes between high and low TGF-β levels was observed to be statistically significant ($p<0.05$).The relationship between TGF-β levels and packed cell volume was negatively correlated ($r = -0.27$).

Conclusion

These findings suggest that fine mechanisms regulate the interaction between TGF-β and IL-8 in the immune response to *Plasmodium falciparum* infection, seemingly directing *in vivo* modulations in red cell population, and indicating a likely pointer to malaria disease and levels of parasitemia.

Keywords: Interleukin-8 (IL-8); Transforming growth factor beta (TGF-β); *Plasmodium falciparum*

INTRODUCTION

Half the world's population of about 3.4 billion people are at risk of malaria. Current figures estimate that about 135-287 million people suffer from malaria and 0.5-0.8 million die annually[1]. In tropical countries especially sub-Saharan Africa, malaria wields great influence on human health where it poses the greatest impact of morbidity and mortality among infectious diseases[2]. In this region of the world severe malaria anemia happens to be a frequent complication of *Plasmodium falciparum* infections in young children and is one of the main causes of severe anemia, with a case-fatality rate reaching 23% in malaria holoendemic areas[3]. In a country like Nigeria which still remains one of the two countries accounting for 40% of global

malaria deaths[1] (WHO, 2013), there still remains a high prevalence of anemia and malaria parasitemia in the general population especially in malaria endemic areas[4].

Malaria is highly endemic in Lagos state posing a major challenge to the state as it impedes human development. The cosmopolitan nature of the state, coupled with the abundant distribution of coastal areas, encourages the development of stagnant water which fosters the breeding of insect vectors and the rise in malaria[5]. The Ikorodu area currently has a prevalence rate of 13% compared to 0.5% prevalence on the Lagos Island[6].

Complications of severe anemia and cerebral malaria are the major cause of morbidity and mortality, although evidence suggests that the host's immunological response plays a vital role in the pathophysiology of this disease in humans[7,8]. Immunity to malaria is dependent on both the innate and adaptive arms (both cell- and antibody mediated) of the immune system, which are required for adequate protection[9]. Regulation of the host-immune response to invading pathogens depends largely on the development of acquired immunity mediated by pro- and anti-inflammatory cytokines[10].The pathogenesis of the complications of anaemia and parasitemia in *P. falciparum* infections appears to involve a dysregulation of this immune system[11]. The balance between pro- and anti-inflammatory cytokines and the pathogenic effects that result from dysregulation still however remain poorly understood[12].

The pathogenesis of malaria is a complex process in which a common outcome might be reached by different routes[11]. This idea emphasizes the relevance of diagnostic and prognostic parameters in predicting the specific risks associated with different clinical characteristics. Of relevance to diagnosis and prognosis is the perception that specific clinical conditions might have distinguishable immunological features. It has been suggested that a marked imbalance in cytokines found in serum might be used as a marker of progression to fatal outcome[13].

The pathology associated with malaria, being immune mediated and capable of leading to adverse systemic effects, further engenders the quest in the determination of the roles and relationships of associated cytokines which may be linked with heightened disease severity during the presentation of various malaria parasites stages[14].

The goal of improving malaria therapy and furthering advancements in the design of effective vaccines may thus be achieved with a better understanding of the workings of these cytokines produced *in vivo* during parasite killing. Attention has also focused on blocking cytokines which are harmful to the host, particularly during overwhelming infection.

Interleukin-8 (IL-8) belongs to a class of cytokines referred to as chemokines. Chemokines are small pro-inflammatory peptides (8 to 17 KDa) which play important roles in bridging the innate and adaptive immune systems[15] (Luster, 2002). IL-8 is secreted by many cells including monocytes, macrophages, and endothelial cells. It is chemotactic for T cells and is a chemoattractant and an activator of neutrophils, facilitating the passage of these leukocytes and other cells from the circulation into the tissues[16, 17]. They do this by activating corresponding receptors on responsive cells, thereby inducing chemotaxis of immune cells to sites of infection; activated neutrophils in tissues degranulate and cause tissue damage.

Several studies implicate IL-8 among other cytokines in the pathogenesis of severe malaria cases compared to uncomplicated and matched healthy controls[12, 18]. Some chemokines, including IL-8 have also been identified as biomarkers of cerebral malaria mortality in Ghanaian children[19]. Furthermore, reports from Thailand have revealed elevated IL-8 levels in falciparum-infected patients[16]. IL-8 serum concentration among patients suffering from severe *P. falciparum* malaria have been shown to be highest at a time when no parasite was detected in the blood smear[20], indicating a continued production of bioactive IL-8 during and after clinical recovery from *P. falciparum* infection, which may suggest that this

cytokine is involved as well in the healing process[16]. There is however still a dearth of *in vivo* data on IL-8 in clinical infections in our locality.[18]

Transforming growth factor (TGF-β) is a multifunctional growth factor peptide reported to be involved in many physiological and pathological processes such as vascular remodeling and atherogenesis[21]. TGF-β, produced by a wide range of cells such as macrophages, NK, T, and B cells, has a pivotal role in the control of the transition between pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type) response during the acute and resolving phases of malaria infection[22]. The balance between Th1 and Th2 immune response is important in determining the level of malaria parasitemia, disease outcome and rates of recovery, the over production of both pro-inflammatory and anti-inflammatory cytokines can be responsible for disease severity and mortality[23].

TGF-β appears to be an important cytokine for maintaining the balance between protection and progression towards disease, depending on the plasma concentration. At low levels, TGF-β has pro-inflammatory properties, whereas high levels of TGF-β are associated anti-inflammatory effects[24]. Although pro-inflammatroy responses often are associated with protective cell-mediated immunity, and anti-inflammatory responses are associated with susceptibility to malaria, the balance of pro- and anti-inflammatory cytokines appears to be an important determinant of whether a protective or a pathogenic response develops.[25]

In *P. falciparum* malaria the involvement of TGF-β becomes necessary since it inhibits IFN-γ and TNF-α production, cytokines which support the inflammatory process, at the same time up-regulating IL-10 and down-regulating the expression of adhesion molecules. Two important roles of TGF-β in malaria infection are thus identified depending on the phase of the infection; promoting Th1-mediated mechanisms that control parasite growth early in the infection and down regulating Th1-like responses to limit inflammation associated pathology later in the infection[24]. The overall outcome of both phases being likely periods of an experienced pathological condition during the course of the malaria disease. It is thus apparent that the timing and magnitude of the TGF-β response is crucially important in determining the outcome of infection[26].

In a longitudinal study of the relationship between pro- and anti- inflammatory cytokines production and clinical immunity to malaria in Ghana, it was shown that high ratios of IFN-γ, IL-12 or TNF-α to TGF-β were associated with reduced risk of parasitemia but increased risk of febrile illness[27]. These data support the notion that anti-inflammatory cytokines are required to down-regulate the pathological effects of high concentrations of pro-inflammatory cytokines. A dynamic equilibrium seems to be required with pro-inflammatory effector mechanisms targeting and controlling the parasite, and anti-inflammatory cytokines suppressing immunopathology.

In Lagos, few studies have examined the immune response in *P. falciparum* malaria to determine the relationship between plasma cytokine levels and parameters relating to disease severity in the host, especially in areas where malaria is highly endemic, such as Ikorodu coastal areas of Lagos state. The main goal of his study therefore, was to measure the plasma levels of IL-8 and TGF-β and to assess the relationships between these cytokines and various host factors pertaining to malaria disease severity in our environment.

MATERIALS AND METHODS

Study area

This study was conducted at a General hospital and four primary health centres at Ikorodu Local Government Area, located approximately 18.5 km from the state capital Ikeja, in Lagos State Nigeria. Ikorodu, a city with an area of 394 sq.km and one of 57 Local Government areas in Lagos state, is located in the southwestern corner of Nigeria close to the Gulf of Guinea, on the Northern edge of the Lagos lagoon; the city shares a boundary with Ogun state.

The General Hospital was at Ijede and the primary health centres were at Imota, Bayeku, Agura and St. Kizito Health Post Oreta.The geographical coordinates

of the sites visited are in close contiguity, with Ijede 6° 34' 0" North, 3° 36' 0" East; Agura 6° 34' 0" North, 3° 38' 0" East; Imota 6° 40' 0" North, 3° 40' 0" East; Bayeku 6° 44' 0" North, 3° 41' 0" East; and Oreta 6° 53' 0" North, 3° 52' 0"East.Agriculture mainly farming and fishing are their major occupations, although with economic expansion, these communities now boast of a variety of vocational enterprises, besides salary earning jobs. This Local Government area had previously been identified as highly endemic for malaria transmission in the state[28].

The location of these communities by the shore of Lagos lagoon and around coastal areas encourages the development of stagnant water responsible for the breeding of anopheles mosquito, a situation contributing to the stable pattern and continuous transmission of malaria all year round

Study design

An analytical, cross-sectional study design was utilized.

Study population

A hundred and thirty six individuals who had malaria attack based on *P. falciparum* parasitaemia in their thick blood smears stained by Giemsa stain participated in the study. They also had fever (axillary temperature of >37,5°C) and clinical symptoms such as headache vomiting, prostration and other symptoms and signs of malaria. They were recruited after informed consent was obtained following a thorough explanation of all procedures and the objectives of the investigation.

Ethical consideration/issues

Institutional: Prior to the commencement of sample collection, ethical approval from the College of Medicine Ethics Committee of University of Lagos and Lagos State Health management Board was obtained.

Individual: Patients were evaluated and enrolled at the health centers. A written informed consent for participation in the study was obtained, while an assent form was obtained from parents or guardians of individuals considered as minors prior to inclusion in the study.

Patients were informed of the procedures involved in the study; with regards to risk/harm they were informed of the discomfort associated with the collection of blood samples, no other adverse effects or risk was expected to be associated with participation in this study.

Patients were free to consent or decline from the study at any time during the study period. Patients found positive for malaria were given adequate treatments, according to the national malaria treatment guidelines.

Sample collection

Whole venous blood (3 ml) was collected from a peripheral vein by venipuncture into sterile EDTA vacutainer tubes. The blood was processed by centrifugation and afterwards the plasma was stored at -70°C.

Parasitological assessments

Blood samples were stained with 3% Giemsa's solution for 45minutes. Diagnoses were established by standard light microscopy. The thick blood smears for the initial screening were examined for the presence of parasitemia in a limited number of microscopic fields. A second blood smear was used to calculate the parasite burden. The level of parasitemia (asexual parasites/µL blood) was estimated from the thick smears by counting the number of asexual parasites against leucocytes, assuming each patient had 8000 leucocytes/µL. Parasitemia per µL was calculated by using the formula: (Parasitemia (per µL) = number of parasites X 8000/number of leucocytes).

Cytokine assays

For cytokine assays, venous blood samples were drawn aseptically into Vacutainer® tubes (Beckton Dickinson and Company). Plasma samples were separated and aliquots were frozen at -70°C until assayed. Plasma samples were analysed for IL-8 and TGF-β by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions (MABTECH AB, Sweden). Each plate included a standard curve of

recombinant human cytokine. All specimens were measured in duplicate using an ELISA microplate reader (TECAN sunrise ELISA reader), and the means of the values were used in all analyses.

Statistical analysis

Data was presented as mean ± standard deviation. Student's t-test was used to test the difference between two means, while one-way ANOVA was used to compare means of three or more groups (multiple comparisons of means). Pearson correlation (for parametric data) and Spearman correlation (for non-parametric) were used to test relationship between two variables. Data were analysed with Excel 2007 and SPSS statistical package (SPSS version 17.0, SPSS INC Chicago, IL, USA).A two-tailed *P*value less than 0.05 was considered statistically significant.

RESULTS

Patients

Between 5th August and 20th of November 2013, 1,414 subjects from the primary health centres at Ikorodu Local Government Area of Lagos State, Nigeria were screened for malaria infection. Of these, 136 (9.6%) were smear positive for *P. falciparum* infection. All smear-positive cases met the inclusion criteria for the study and were enrolled in the study. Table 1 shows the baseline characteristics of the malaria patients.

The mean age of the malaria patients was 16.1 years (range: 3-63). At study entry, the mean axillary temperature was 37.3°C (33.5-40.4), while mean parasite density was 35,057.5 (19-245,647) parasites/µL, mean white blood cell count was 5.8 $10x^3/mm^3$ (1.5-24.2x$10^3/mm^3$), mean PCV was 34.2% (12-53%), mean TGF-β was 23,672pg/ml (0-93,675.2pg/ml) while mean IL-8 was 508.8pg/ml (0-3406.6pg/ml).

Difference in plasma cytokine levels and host characteristics

Plasma IL-8 at low levels (< 675.7pg/ml), showed elevated mean parasite densities (43,200±75,819.1parasites/µL) when compared to high levels of IL-8 (> 675.7pg/ml), which had a reduced mean parasite densities (21,097±24,340.4parasites/µL). The difference in mean parasite densities between high and low values of IL-8 was statistically significant ($P < 0.05$). TGF-β at high and low levels did not show any significant difference with respect to parasite densities of the malaria patients (Table 3).

Among malaria patients with low levels of TGF-β (< 9,393.6pg/ml), mean values of packed cell volume were seen to be raised within average normal ranges (34.5±8.0), whereas patients with high levels of TGF-β (> 9,393.6pg/ml), had mean values of packed cell volume being lowered (29.2±12.5). The difference in mean values of packed cell volume between high and low levels of TGF-β was observed to be statistically significant ($P < 0.05$). There was a negative correlation between TGF-β levels and packed cell volume ($r = -0.27$, $P = 0.001$). IL-8 did not show any correlation with this parameter (Table 4).

DISCUSSION

In a bid to developing new approaches and methods of controlling severe disease due to malaria a better understanding of malaria pathogenesis is of vital importance.Cytokines play a significant role in the progression and outcome of malaria[22, 29-31]. Certain complications such as that of anemia and cerebral malaria, predominantly seen in African children are known to be major causes of morbidity and mortality in malaria disease[29]. In this study, the packed cell volume, a test used to screen for anemia[30], was found to be negatively correlated with TGF-β.

The pathogenesis of malaria anemia is not well understood, although destruction of infected erythrocytes accompanied by clearance of uninfected erythrocytes, erythropoietic suppression and dyserythropoiesis, can all contribute to anemia[3]. Various cytokines have been implicated in these effects although their role in the development of anemia is not well understood[12]. Earlier research had reported malaria-induced anemia as being multifactorial, with hemolysis occurring more frequently in nonimmune children and dyserythropoiesis occurring more often in regions with frequent and recurrent infections[29, 12].

Other research have also implicated TGF-β as being a powerful inhibitor of erythropoiesis, acting essentially by decreasing recruitment of hematopoietic progenitors from quiescence into active cell-cycling status and by increasing the length of the G1 phase of cycling progenitors[31-33].

Murine studies have now demonstrated that elevated TNF-α levels contribute to bone marrow suppression and red cell destruction whereas elevated IL-10 is thought to stimulate hematopoiesis[12]. TNF-α elevation has been associated with anemia and high density *P. falciparum* infection in children[34], whereas reduced IL-10 [35], and IL-10/TNF-α ratios have been demonstrated in African children with severe malaria-induced anemia [36-39].

TGF-β is known to down-regulate the production of TNF-α and IL-10 and has been attributed to both positive and negative effects on erythropoiesis[38, 31, 40]. Recent studies have however made it clear that an important cause of reduced erythropoiesis in children with severe malaria anemia is due to an imbalance in inflammatory mediatior[39].

TGF-β, a protein that controls proliferation, cellular differentiation, and other functions in most cells, has a crucial role in controlling the transition between pro-inflammatory (Th1-type) and anti-inflamatory (Th2-type) responses during the acute and resolving phases of malaria infection[41,42].

A successful type 1 response to malaria requires a well-timed and proportional release of interleukin IL-12, IFN-γ, and TNF-α to minimize parasitemia and preserve erythropoiesis[39]. IL-12 stimulates production of IFN-γ and TNF-α from T-cells and natural killer (NK) cells, thereby further augmenting type 1 responses. A number of cytokines and chemokines can promote IL-12 [e.g, granulocyte macrophage-colony stimulating factor (GM-CSF) and IFN-γ], while others decrease IL-12 production [e.g, TGF-β, IL-13, monocyte chemotactic protein (MCP)-1/CCL2]. As such, the overall ability of the innate immune response to generate IL-12 a condition largely determined by the timing of TGF-β production is an important event that mediates the development of malaria anemia[39].

In our study, low mean volumes of packed red cells, conditions indicative of anemia, were associated with TGF-β at high concentrations. Earlier reports postulated that the anti-inflammatory response of TGF-β may suppress IL-12 but may be insufficient for preventing excessive TNF-α production, which could promote anemia in children with acute malaria[10]. Also malaria patients with slightly raised and normal volumes of packed red cells, a state deviating from an anemic conditionwere associated withlow TGF-β concentrations.This was in agreement with studies done in Gabon and Thailand where severe malaria, described as being characterized by high-density parasitemia and severe anemia, had reduced TGF-β levels being linked to severe malaria[23, 43, 10]. However, in contrast, a study conducted in the city of Ouagadougou (Burkina Faso) found elevated TGF-β levels in children with severe malaria[22]. These variances in results were explained as being due to the differences in malaria endemicity in both rural and urban regions of Gabon and Burkina Faso respectively where studies were conducted. The rural area of Lambaréné, Gabon had a high level of *P. falciparum* transmission, whereas the urban region of Ouagadougou is mesoendemic for *P. falciparum*[39]. This close similarity in endemicity shared between our study site at Ikorodu, with its proximity to coastal areas and all year round transmission, and that seen in Gabon, explains the similarity in results between these two sites.This further buttresses the contention by Lyke et al, which asserts the frequency of malaria-induced dyserythropoiesis occurring more often in regions with frequent and recurrent infections[12]. In our study, TGF-β levels were however not associated with the number of parasites; this is in accord with previous research done where no correlation between parasitemia and TGF-β levels could be found[43].

IL-8 being a neutrophil chemoattractant, performs pro-inflammatory functions and thus should play a significant role in malaria disease outcome. Little is however known about its role in the pathogenesis of malaria[44], with insinuated opinions about this role being

unlikely[12]. We reported mean parasitemia levels to be elevated in patients with low levels of IL-8 and reduced mean parasitemia levels in patients who had high IL-8 plasma concentrations, these findings however diverge from previous reports, where higher levels of this cytokine were reported to be found mainly among malaria patients with severe parasite burden[45, 18]. Studies in Thailand provided reports where IL-8 was seen to be elevated in severe non-fatal malaria patients[16]; similar results were also observed in Malian children [12], although children with severe malaria in this study had ten-fold higher concentrations of IL-8 compared to either healthy controls or individuals with uncomplicated malaria, suggesting that geographical placements may have an influence on cytokine levels in relation to parasitemia. Furthermore, in contrast to studies reporting a correlation between IL-8 and parasite number, we found no such relationship between these two parameters[20]. We however observed initial mean plasma IL-8 concentrations being raised at 508.8 pg/ml in malaria positive patients tested, whereas in subjects without malaria, no plasma IL-8 was detectable, this result has been found to be in line with previous studies where levels of endogenous pyrogens such as IL-8 were reported to have been elevated in malaria, with patients devoid of malaria, having no plasma IL-8 detectable[16]. An interesting find in the study by Friedland et al, was that plasma IL-8 levels remained elevated throughout the period of investigation[16]; the continued production of IL-8 during and after clinical recovery from *P. falciparum* infection was believed to indicate that the cytokine is involved in the healing process, which may explain the increased IL-8 levels having a mean reduced parasitemia in our study, indicating a likely curative function attributed to this cytokine. Nonetheless, additional studies in Gabonese children and adults did however illustrate that higher plasma IL-8 levels were associated with acute malaria and a slow cure after malaria chemotherapy[45]. Consequently, we postulate that elevation in plasma concentration of this chemokine in *in vivo* circulation is dependent on the stage of the malaria disease in the host, whether at initial onset or at a future state towards resolution of infection.

CONCLUSION

Studies outlined here support a model in which the pathogenesis of malaria disease is largely driven by an over production or an underproduction of anti-inflammatory cytokines and chemokines and suppression of erythropoiesis that is driven by dysregulation of innate anti-inflammatory mediators. With no definite end in sight to having the upper hand to challenges posed by malaria infections, it is our confident desire that advancing in malaria disease prognoses be continually improved on particularly in areas directed to anemia and parasite burden as these are major concerns in morbidity and mortality amongst African children.

Further work in different geographic areas is needed to confirm whether these cytokines have similar effects on the levels of parasitemia and packed cell volumes in different populations.

The design of intervention strategies will boarder around interests directed towards cells and cytokines possibly implicated in dyserythropoiesis or erythropoietic suppressionin malaria mediated anemia, as well as biomarkers for indications for parasitemia of different degrees.

Since there is a dearth of information, it is therefore recommended that data in this direction be investigated in order to establish their roles in malaria disease pathogenicity, especially at varying stages of malaria disease states. Further studies are therefore, needed to better understand the associations between parasitic burden and balancing effects of pro- and anti-inflammatory cytokines in determining resolution or persistence and worsening of *P. falciparum* malaria infection.

REFERENCES

1. World Malaria Report. Geneva, World Health Organization, 2013. http://www.who.int/malaria/world_malaria_report_2013/en
2. World Health Organisation (2000).Severe falciparum malaria. *Trans. R Soc. Trop. Med. Hyg. 2000; I94:* (Suppl. 1): Si-S90.
3. Boeuf PS, Loizon S, Awandare GA, Tetteh JKA, Addae MM, Adeji GO, Goka B, Kurtzhals JAL,

Puijalon O, Hviid L, Akanmori BD and Behr C. Insights into deregulated TNF and IL-10 production in malaria: implications for understanding severe malaria anemia. *Malaria Journal. 2012;* 11:253.

4. Agan TU, Ekabua JE, Udoh AE, Ekanem EI, Efiok EE and Mgbekem MA. Prevalence of Anaemia in Women with Asymptomatic Malaria Parasitemia at first antenatal care visit at the University of Calabar Teaching Hospital, Calabar, Nigeria. *Int J Womens Health*. 2010; 2: 229-233.
5. Idris J, Agbe-Davies A, Olugbile O, Taiwo O, Bakare O and Arowolo T. Malaria control activities in Lagos (2000-2010): A review. 2011. Retrieved from www.lsmoh.com
6. Anugwara B. Indoor residual spraying: Lagos expands scope to reduce malaria scourge. Retrieved from; 2014; www.mydailynewswatch.ng
7. Plebanski M, Hill AVS.The immunology of malaria. *Curr Opin Immunol. 2000;* **12:** 437–41.
8. Luty AJ, Perkins DJ, Lell B. Low interleukin-12 activity in severe *Plasmodium falciparum* malaria. *Infect Immun. 2000;* **68:** 3909–15.
9. Perlmann P and TroyeBlornberg M. Malaria and the Immune System in Humans. *Chem Immunol. 2002;* 80:229-242.
10. Perkins DJ, Weinberg JB and Kremsner PG. Reduced interleukin-12 and Transforming Growth Factor-β in severe Childhood Malaria: Relationship of Cytokine Balance with Disease Severity.*J. Infect. Dis.* 2000; 182:988-92.
11. Miller LH, Baruch DI, Marsh K and Doumbo OK. The pathogenic basis of malaria. *Nature. 2002;* 371:508-510.
12. Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, Kone A, Harley R, Plowe CV, Doumbo OK, Sztein MB. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe *Plasmodium falciparum* malaria and matched uncomplicated malaria or healthy controls. *Infec Immun. 2004;* 72:5630-5637.
13. Angulo I and Fresno M.Cytokines in the Pathogenesis of and Protection against Malaria.*Clinical and Diagnostic Laboratory Immunology.* 2002; 9(6):1145-1152.
14. Omer FM, De Souza JB and Riley EM. Differential induction of TGF-β regulates pro-inflammatory cytokine production and determines the outcome of lethal and nonlethal *Plasmodium yoelii* infections. *J. immunol. 2003a;* 171: 5430-5436.
15. Luster AD.The role of chemokines in linking innate and adaptive immunity. *Curr.Opin. Immunol. 2002;* 14:129-135.
16. Friedland SJ, Ho M, Rennick GD, Bunnay D, White NJ and Griffin GE.IL-8 and *Plasmodium falciparum* malaria in Thailand. *Trans. R. Soc. Trop. Med. Hyg. 1993;* 87: 54-57.
17. Dinarello CA. Proinflammatory Cytokines. *Chest*; 2000; 118(2):503-508.
18. Nmorsi OPG, Isaac C, Ukwandu NCD, Ekundayo AO, Ekozien MI and Eifediyi RA. Interleukin-8 (IL-8) profile in Nigerians with *Plasmodium falciparum* infection. *Report and Opinion. 2009*; 1(2):73-77
19. Armah HB, Wilson NO, Sarfo BY, Powell MD, Bond VC, Anderson W, Adeji AA, Gyasi RK, Tetty Y, Wierdu KE, Tongren JE, Udhagajumar V and Stiles JK. Cerebrospinal fluid serum biomarkers of cerebral malaria mortality in Ghanian children. *Malaria Journal. 2007;* 6:147.
20. Burgman H, Hollenstein U, Wenisch C, Thalhammer F, Looareesuwan S and Granninger W. Serum concentrations of MIP-1 and Interleukin-8 in patients suffering from acute *Plasmodium falciparum* malaria. *Immunology and immunopathology. 1995;* 17:292-296.
21. Porreca E, Di Febbo C, Baccante G, Di Nisio M and Cuccurullo F. Increased Transforming Growth Factor-Beta1 Circulating Levels and Production in Human Monocytes After 3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A Reductase Inhibition With Pravastatin.*Journal of the American College of Cardiology. 2002*; 39(11)
22. Malaguarnera L and Musumeci S.The immune response to *Plasmodium falciparum* malaria. *The Lancet Infectious diseases. 2002;* 2: 472-478.
23. Musumeci M, Malaguarnera L, Simporè J, Messina A and Musumeci S (2003). Modulation of

immune response in *Plasmodium falciparum* malaria: role of IL-12, IL-18 and TGF-β. *Cytokine. 2003*; **21:** 172-178.

24. Omer FM, Kurtzhals JA, Riley EM (2000). Maintaining the immunological balance in parasitic infections: a role for TGF-beta? *Parasitol Today;* **16:** 18–23.
25. Rovira-Lallbona E, Moncunill G, Bassat Q, Aguilar R, Machevo S, Puyol L, Quinto L, Menéndez C, Chitnis CE, Alonso PL, Dobaño C and Mayor A. Low antibodies against *Plasmodium falciparum* and imbalanced pro-inflammatory cytokines are associated with severe malaria in Mozambican children: a case-control study. *Malaria Journal. 2012;* 11:181.
26. Omer FM, De Souza JB, Corran PH, Sultan AA and Riley EM (2003b). Activation of Transforming Growth Factor β by Malaria Parasite-derived Metalloproteinases and a Thrombospondin-like Molecule.
27. Dodoo D, Omer FM, Todd J, Akanmori BD, Koram KA, Riley EM. Absolute levels and ratios of proi-nflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to *Plasmodium falciparum* malaria. *J Infec Dis. 2002*; 185: 971-9.
28. Chidiogo E. Government sprays insecticides on 15,000 houses in Lagos. 2011; Article retrieved from www.dailytimes.com.ng
29. Day NP, Hien TT, Schollaardt T, Loc PP, Chuong LV, Chau TT, Mai NT, Phu NH, Sinh DX, Whithe NJ, Ho M. The prognostic and pathophysiology role of pro- and anti-inflammatory cytokines in severe malaria.*J. Infec. Dis.* 1999; 181:176.
30. Cheesbrough M. Packed cell volume and red cell indicies, Heamatological tests. In District Laboratory Practice in Tropical countries part 2, 2nded update. Cape Town South Africa: Cambridge University Press; 2010: 309-313.
31. Zermati Y, Fichelson S, Valensi F, Freyssinier JM, Rouyer-Fessard P, Cramer E, Guichard J, Varet B, Hermine O. Transforming growth factor inhibits erythropoiesis by blocking proliferation and Accelerating differentiation of erythroid progenitors. *Exp Hematol. 2000;* 28(8):885-94.
32. Fortunel NO, Hatzfeld A and Hatzfeld JA.Transforming growth factor-β: plietropic role in the regulation of hematopoiesis. *Blood. 2000;* 96:2022-2036.
33. Dong M and Blobe GC. Role of Transforming growth factor-β in hematological malignancies. *Blood. 2006*; 107:4589-4596.
34. Schaffer N, Grau GE, Hedberg K, Davachi F, Lyamba B, Hightower AW, Breman JG and Phuc ND. Tumor necrosis factor and severe malaria.*J. Infec. Dis. 1991;* 163:96-101.
35. Kurtzhals JA, Adabayeri V, Goka BQ, Akanmori BD, Oliver-Commey JO, Nkrumah FK, Behr C and Hviid L. Low plasma concentration of interleukin 10 in severe malaria anemia compared with cerebral and uncomplicated malaria. *Lancet. 1998*; 351: 1768-1772.
36. May J, Lell B, Luty AJ, Meyer CG and Kremsner. Plasma interleukin-10: tumor necrosis factor (TNF)-alpha ratio is associated with TNF promoter variants and predicts malarial complications. *J. Infec.Dis. 2000;* 182: 1570-1573.
37. Othoro C, Lal AA, Nahlen B, Koech D, Orago AS and Udhayaku V. A low interleukin-10 tumor necrosis factor-alpha ratio is associated with malaria anemia in children residing in a holoendemic malaria region in western Kenya. *J. Infec. Dis.1999;* 179:279-282.
38. GourleyIS, Kurtis JD, Kamoun M, Amon JJ and Duffy PE (2002). Profound bias in interferon-γ and Interleukin-6 allele frequencies in Western Kenya, where severe malaria Anemia is common in children.*The Journal of Infectious Diseases Society of America.*186:1007-12.
39. Perkins DJ, Were T, Davenport GC, Kempaiah P, Hittner JB and Ong'echa JM. Severe malaria anemia: Innate immunity and pathogenesis. *Int. J.Biol Sci. 2011;* 7(9):1427-1442.
40. Hino M, Tojo A, Miyazono K, Urabe A, Takaku F (1988). Effects of type beta transforming growth factors on haematopoietic progenitor cells.*Br J Haematol;* 70(2):143-7.
41. Gowda DC (2007). Pro-inflammatory responses and cell signaling during malaria infection: The parasite glycosylphosphatidylinositol ligand. In:

Denkers E, Gazzinelli R. Protozoans in Macrophages. 15th ed. Chicago: Landes Bioscience; 2007.

42. Portugal S, Moebius J, Skinner J, Doumbo S, Doumtabe D, Kone Y, Dia S, Kanakabandi K, Sturdevant DE, Virtaneva K, Porcella SF, Li S, Doumbo K, Kayentao K, Ongoiba A, Traore B, Crompton PD. Exposure dependent control of malaria-induced inflammation in children. *PLOS Pathogens. 2014*; 10:4
43. Wenisch C, Parschalk B, Burgmann H, Looareesuwan S and Graninger W. Decreased serum levels of TGF-β in patients with acute *Plasmodium falciparum* malaria. *Journal of Clinical immunology. 1995;* 15:69-73.
44. Zeyrek FY, Kurcer MA, Zeyrek D and Simsek Z. Parasite density and serum cytokine levels in *Plasmodium vivax* malaria in Turkey. *Parasite immunology. 2006*; 28: 201-207.
45. Boström S, Giusti P, Arama C, Persson J, Dara V, Traore B, Dolo A, Doumbo O and Troye-Blomberg M (2012). Changes in the levels of cytokines, chemokines and malaria-specific antibodies in response to *Plasmodium falciparum* infection in children living in sympatry in Mali. *Malaria Journal. 2012*; 11; 109.
46. Kremsner PG, Winkler S, Brandts C, Wilding E, Jenne L, Graninger W, Prada J, Bienzle U, Juillard P, Grau GE. Prediction of accelerated cure in *Plasmodium falciparum* malaria by the elevated capacity of tumor necrosis factor production.*Am J Trop Med Hyg. 1995;* 53(5):532-8.

Table 1: Baseline characteristics of patients

Characteristics	Frequency	Percentage (%)
Age (Years)		
3-17 years	94	69.1
18-32 years	27	19.9
33-47 years	11	8.1
48-63 years	4	2.9
Sex		
Male	58	42.7
Female	78	57.4
Temperature(°C)		
<36.5	42	30.9
36.5-37.5	36	26.5
>37.5	58	42.7
White blood cell count ($\times 10^3/mm^3$)		
<4.0	37	27.2
4.0-10.0	88	64.7
>10.0	11	8.1
Packed cell volume (%)		
<35	71	52.2
35-55	65	47.8
Parasite density (parasites/μL)		
<10,000	54	39.7
10,000-19,999	24	17.6
20,000-39,999	22	16.2
40,000-59,999	15	11.2
60,000-99,999	11	8.1
>100,000	10	7.3
Cytokines (pg/ml)		
IL-8 low levels	86	63.2
IL-8 high levels	50	36.8
TGF-β low levels	77	56.6
TGF-β high levels	59	43.4

Table 2: Mean comparison of IL-8 and TGF-β using student t-Test at varying levels of different host parameters Significant at $P \leq 0.05$ (95% confidence Interval)

Body Parameters			N	Mean	Standard Deviation	P – value
PCV levels (%)	<35	IL8	71	406.5	±744.3	0.46
	35-55		65	620.5	±772.9	
	<35	TGFβ	71	29252.5	±33810.5	0.00
	35-55		65	17576.3	±25753.6	
Temperature Levels (℃)	<37.5	IL8	78	499.2	±790.5	0.743
	>37.5		58	521.7	±730.8	
	<37.5	TGFβ	78	25764	±32048.2	0.112
	>37.5		58	20859	±28778.8	
WBC ranges /mm3	<4000	IL8	37	438.4	±701.4	0.633
	4000-10,000		88	554.1	±798.7	
	>10,000		11	382.8	±694.9	
	<4000	TGFβ	37	32137.6	±35515.9	0.108
	4000-10,000		88	21340.4	±28449.2	
	>10,000		11	13849.9	±26607.8	
Age (Years)	3-17 years	IL8	94	501.3	±793.9	0.773
	18 - 32years		27	574.0	±753.3	
	33 - 47 years		11	331.9	±464.9	
	48 - 63 years		4	730.1	±889.7	
	3-17 years	TGFβ	94	21167.4	±28108.9	0.147
	18 - 32years		27	35486.2	±38331.8	
	33 - 47 years		11	20411.5	±29515.3	
	48 - 63 years		4	11749.6	±23499.2	
Malaria Status	Positive for malaria	IL8	136	508.8	±762.9	0.001
	Negative for malaria		5	0	0	
	Positive for malaria	TGFβ	136	23672	±30682.9	0.932
	Negative for malaria		5	35325	±32526.6	

Table 3: Mean comparisons of variable host factors at low and high cytokine levels using student t-Test

	Host factors	Cytokine level	N	Mean	P – value
TGF-β	PCV (%)	Low TGFβ level	77	34.5	0.007
		High TGFβ level	59	29.2	
	Temperature (℃)	Low TGF-β level	77	37.3	0.937
		High TGF-β level	59	37.3	
	Parasite density (Parasites/µl)	Low TGF-β level	77	33,213	0.253
		High TGF-β level	59	37,502	
IL-8	PCV (%)	Low IL-8 level	86	30.7	0.209
		High IL-8 level	50	34.8	
	Temperature (℃)	Low IL-8 level	86	37.3	0.512
		High IL-8 level	50	37.3	
	Parasite density (Parasites/µl)	Low IL-8 level	86	43,200.0	0.007
		High IL-8 level	50	21,097.0	

Significant at $P \leq 0.05$ (95% confidence Interval)

Table 4: Correlation of white cell counts, packed cell volume and cytokine concentration

	WBC count		Packed cell volume		Parasite count	
Cytokines	r-value	p-value	r-value	p-value	r-value	p-value
IL-8	0.003	0.974	0.171	0.044	-0.13	0.125
TGF-β	-0.128	0.131	-0.270**	0.001	0.095	0.263

** Correlation is significant at the 99% confidence interval (CI) $P \leq 0.01$

Challenges in Indigenous Medicine Development in Nigeria

Professor O. K. Udeala, Pioneer Dean, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria.

Excerpts from a public lecture given at the *1st International Malaria Colloquium: Applying Molecular Techniques to Intervention in Malaria Elimination in Nigeria, University of Port Harcourt Rivers. May, 2006.*

INTRODUCTION

- The discovery of medicinal substances for treatment of human ailments has been described as ``hit or miss'' in humans
- Quinine and digitalis were accepted as effective medications while majority of the plant extracts are now dubbed placebos; this may be going too far!
- Inhibition of O. gratissimum leaf extracts for prostate cancer cells (PC3-AR) in a modern USA research laboratory as attested by multinationals may contest this opinion.
- Synthesis of salvarsan by Ehrlich at the turn of the 20th century marked the transition of plants/their extracts to synthetics in the laboratory.

SYNTHESIS ERA

- The synthesis of sulpha medicines in 1930 added to the drive to produce pure chemical substances in the laboratory
- Alexander Fleming in 1929 discovered that small amount of substances produced by Penicillium mold were effective against bacteria
- This added drive for screening several microbes for antibiotics
- World War II, and the need for new medications compelled collaboration between pharmaceutical companies and chemists

DECADE FOR INFECTIOUS DISEASE (1960 – 1970)

- The focus was on infectious diseases
- Biomedical scientists got busy purifying extracts from plants from which derivatives or analogues were made to increase potency
- Other new technologies such as Biotechnology and Genetic Engineering came into pharmaceutical science
- Biotechnical approaches made possible amount of proteins available for study and development of medical substances and agents
- The expected dividend was to increase the quantity of clinically effective medicines in use

COMBINATORIAL CHEMISTRY

- Synthesis failed to increase clinically effective medicines in the market but combinatorial chemistry was expected to
- Thus came combinatorial chemistry, a rapid reaction of mutually reactive chemical fragments; it is also a version of resin-based ``split-and-mix'' diversity generation and a collection of technologies as well as methods
- High throughput screening (HTS) and bioassay of the analogues or targets left the role of pharmacology undiminished.

BACK TO THE PRIMODIAL

- We are not in the league of combinatorial chemistry, a technology that encompasses several other technologies
- We are also not in the important league of Biotechnology and Chemical Engineering
- We therefore resort to alternative resources since fine chemical industries do not exist
- phytochemicals which have been dubbed placebos become our resource base

CHALLENGES IN INDIGENOUS MEDICINE DEVELOPMENT

- Separation of the actual from *hocus-pocus*
- Infrastructure
- Data Base for Medicinal Plants
- Sustainability of identified resources
- Collaboration, the Key to Success
- National Policy driven Indigenous Medicine Development

SEPARATION OF THE ACTUAL FROM *HOCUS-POCUS*

- Chemical identification of the isolate and proven activity in animal
- Attestation of chemistry and purity of isolate as well as collation by competent and experienced scientists
- These must be in well-equipped laboratory run by competent and qualified personnel
- There is dearth of this calibre of qualified personnel
- One stands the danger of getting *hocus-pocus* than the real thing

INFRASTRUCTURE

- There are some here that have seen well equipped laboratories in their careers
- A modern laboratory should have continuous water supply and get its water purified for use
- Electric power supply is essential; research is not done in candle light
- Researchers send their samples miles away for results; this means the laboratory is not well equipped and younger generation may learn little
- This is the story of our infrastructure and limits how far we can go.

DATABASE FOR MEDICINAL PLANTS

- Some Colonials left a legacy in books of plants used by Traditional Healers
- Such record was made possible by the author's interaction with the healers
- Some laboratories in Nigeria still work with traditional healers and continue to find out more
- A Data Base of molecules that are convertible to potential indigenous medicines will make the building block of Zonal /National Herbarium.

SUSTAINABILITY OF IDENTIFIED PLANT RESOURCE

- Sustainability of medicinal plants accorded a place in the Herbarium require massive multiplication and cultivation
- The ultimate aim is that the harvest should fill cargo ships and feed industries
- This was the case in the hay day of Palm Oil Industry
- The Chemists will have abundant materials to produce needed medicines or analogues.

COLLABORATION CAN BE THE KEY TO SUCCESS

- Collaboration with others with varying expertise can bring success quickly
- Development of medicines is a long term process from the beginning to the end
- Those that are in this business should be encouraged to collaborate with others backed by their institution
- Lack of collaboration does not facilitate solution to problems; Nigerians often want to go it alone!

LAYMAN'S VIEW OF MEDICINE DEVELOPMENT

- The herbalist brings what he believes is a cure for *Ebola* and shows up a week after confident he becomes a nouveau riche
- The corollary is a Nigerian billionaire will not invest in this venture knowing that it might take ``a life time'' to have a billion drop into his account
- Investment in this area is out of the question for entrepreneurs; they are for a quick brew.

NATIONAL POLICY ON INDIGENOUS MEDICINE DEVELOPMENT

- Where are the visionary Leaders who can perceive far ahead the good for this Country?
- The Ship of Governance can become a flotsam; even a flotsam can end on a good landing
- But a way out is Revenue Derivation for a National Committee for Indigenous Medicine Development (NCIM)
- Alas, the Leaders are not yet born!

REFERENCES

1. Charles G. Smith. .*Introduction in the Process of New Drug Discovery and Development.* Second Edtn p 1- 2; Eds C.G. Smith and J.T. O'Donnell, Pub: informa healthcare; 2006, New York London
2. Ekenwe *et al: The effect of O. gratissimum leaf extract on prostate cancer cells.* J. Healthcare Poor Underserved, 2013; 24(4 Suppl.) pp.61 – 9.
3. Michael Rabinowitz and Nigel Shankley (2006) 4. *The Impact of Combinatorial chemistry on Drug Discovery.* Process of New Drug Discovery and Development. Second Edtn p 1- 2; Eds C.G. Smith and J.T. O'Donnell, Pub: informa healthcare; 2006, New York, London. *Pp. 65.*

Profile of the Centre for Malaria Research and Phytomedicine

Introduction

The Centre for Malaria Research and Phytomedicine (CMRAP) was set up by the 7th Vice Chancellor of the University of Port Harcourt, Prof JA Ajienka, to achieve malaria control and elimination through research. Originally conceptualized as a Malaria Research Laboratory, sponsored by the World Health Organisation (WHO/TDR) and the Multilateral Initiative on Malaria in Africa (MIM) in 2001, the purpose of was to carry out phyto-medicinal studies aimed at the identification and clinical evaluation of potential anti-malarial components from the Nigerian phytomedicine compendium. Following the re-designation of the Malaria Research Laboratory and approved by the Governing Council of the University of Port Harcourt in December, 2010, the Vice Chancellor, Prof. J.A. Ajienka, appointed Prof. Omotayo Ebong as the Pioneer Director and Prof. C. A. Nwauche as Assistant Director for CMRAP in March, 2011. Prof. C.A. Nwauche succeeded Prof. Ebong as Director in April 2015 and was himself succeeded by the current Director, Prof. I.M. Siminialayi on August 1, 2016. Dr. Hamilton Opurum was appointed Assistant Director in 2015 and was replaced by Dr Balafama Alex-Hart in 2018.

CMRAP is responsible for leading research and coordinating research activity in the University of Port Harcourt aimed at finding effective and sustainable means of ending malaria in Nigeria in particular but related diseases as well.

The offices of CMRAP are located on the 2nd floor of the right wing of the School of Basic Studies Building at the University Park, University of Port Harcourt, Port Harcourt.

Mission

To achieve malaria control and better health for all through research

Vision

To be a leading Centre of excellence in malaria research and related diseases in Africa

Goal

Contribution to elimination of malaria in Nigeria by 2020.