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Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. J. Cell. Mol. Med. 7:171-178.

Booth M, Bundy DA, Albonico P, Chwaya M, Alawi K (1998). Associations among multiple geohelminth infections in school children from Pemba Island. Parasitol. 116: 85-93.0.

Fransiscus RG, Long JC (1991). Variation in human nasal height and breath, Am. J. Phys. Anthropol. 85(4):419-427.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

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Case Report

Congenital glucose-galactose malabsorption: A rare cause of chronic diarrhea

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Diarrhea present initially at early neonatal period is rare and is generally caused by congenital malabsorptive disorders. Congenital glucose-galactose malabsorption (CGGM) is a rare autosomal recessive disorder present as a protracted diarrhea in early neonatal life. A 3 month-old female infant present with chronic diarrhea, severe failure to thrive, hypernatraemic dehydration and nephrocalcinosis was studied. Early onset diarrhea in a patient with consaguionus parents should alert the pediatricians to think about a rare congenital cause of chronic diarrhea that can present with a life threatening condition.

Key words: Glucose galactose malabsorption, chronic diarrhea in infancy, congenital.

INTRODUCTION

Diarrhea with an onset in the early days of life is rare and generally caused by congenital malabsorptive disorders (Guarino and De Marco, 2004). Congenital glucose-galactose malabsorption (CGGM) is a rare autosomal recessive disorder, which presents as a chronic refractory diarrhea in early neonatal life. It is due to a defect in sodium-coupled transport of glucose and galactose in the enterocyte (Abad-Sinden et al., 1997; Pascual et al., 2004).

In the Arab countries where consanguineous marriage is quite common, CGGM appears to be a common problem as compared to the western populations. There is no data about the incidence or the prevalence of this problem in the Arab world. Lebenthal et al. (1971) have reported an Iraqi adult with GGM and subsequently Abdullah et al. (1996) reported 8 Arab children with a similar problem in Saudi Arabia. Assiri et al. (2013) published five Arab children with GGM and one of them developed gangrene of both legs as a complication of hypernatremia and dehydration, necessitating bilateral amputation. Two infants had nephrolithiasis (Assiri et al., 2013).

This study is presents an infant with chronic diarrhea, hypernatraemic dehydration and nephrocalcinosis, who was diagnosed as congenital GGM.

CASE REPORT

A 3 months old Saudi girl presented to pediatric emergency department was with the history of diarrhea since age of 2 days. She was a product of full term, normal delivery for primigravida mother with an uneventful pregnancy, with birth weight of 2.4 kg. At the second day of her life she started to have watery diarrhea around 10 times per day, yellowish in color not associated with mucous or blood with the history of abdominal distention and poor weight gain. No history of vomiting, skin rash, lethargy or poor feeding. There was no history of repeated infection.

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Figure 1. Severe muscle wasting and malnutrition.

She was admitted at the age of one week at local hospital for 5 days, treated as acute gastroenteritis and was discharged with little improvement. Another admission at the age of 2 months to private hospital investigated and was treated for 20 days with no improvement. She received birth vaccine, her development was appropriate to her age. She was the first baby of first degree cousin parents.

On examination, she looks unwell, conscious, pale, not cyanosed, cachectic, not on respiratory distress with moderate dehydration, no dysmorphic feature, and normal vital signs. Her growth parameters were below third percentile and the weight for age was 42%. She was with scanty hair with normal color, anterior fontanel, open and depressed measuring of 2 × 1.5 cm.

She has diminished subcutaneous fat at the mid-arm with reduced muscle bulk at the gluteal region. She has mild abdominal distention with unremarkable systemic examination (Figure 1). Blood investigations showed serum sodium of 173 mmol/L, potassium of 4.2 mmol/L, urea of 223 mg/dl, creatinine of 0.9 mg/dl and pH of 7.20 and HCO_3 of 11.6 with base excess of -16.1. Stool was acidic and showed no ova or cyst. No stool sugar chromatography was available. Ultrasound abdomen showed nephrocalcinosis in both kidneys (Figure 2). She

was admitted to pediatric ward, tried on many formulae, including lactose free, semi elemental and elemental formula with no change in diarrhea. Metabolic acidosis and pre-renal azotemia were treated with appropriate intravenous fluid. Patient kept NPO with marked improvement of diarrhea. The diagnosis of CGGM was contemplated based on the history of early onset of acidic osmotic diarrhea causing hypernatraemic dehydration that did not improve with elemental diet. The patient was started on galactomin 19 formula which was glucose-galactose free formula (fructose based formula), after the formula was started, the patient showed dramatic improvement, her diarrhea stopped, urea and electrolyte 2 days after introduction of formula was normalized: urea 31.9, creatinine 0.10, Na 134, and K 3.9. The patient was discharged with diagnosis of CGGM on galactomin 19 formula. After four weeks, she presented to gastroenterology clinic with weight of length, good muscle bulk and subcutaneous fat (Figure 3).

DISCUSSION

Differential diagnoses of any patient with early onset neonatal diarrhea are challenging, but fortunately, it has

Figure 2. Ultrasound abdomen showed bilateral nephrocalcinosis.

Figure 3. The patient 6 weeks after starting galactomin 19 formula.

limited causes which include congenital microvillus atrophy, tufting enteropathy, congenital GGM, congenital lactase deficiency, congenital malabsorption of chloride and sodium, bile acid malabsorption, and congenital enterokinase deficiency (Guarino and De Marco, 2004). CGGM is a rare autosomal recessive disease (Martin and Wright, 2004), first reported in Sweden and France by Linquist and Meeuwisse (1962). Currently, only about 300 cases have been reported worldwide (Martin and Wright, 2004). There are scattered cases reported in the neighboring countries like Oman and Malaysia (Lee et al., 2009; Al-Lawati and Vargees, 2008). In our country, thirteen cases have been reported in Saudi Arabia and all of them were presented with an early protracted diarrhea (Abdullah et al., 1996; Assiri et al., 2013), and this is the first case reported in southwest region of the Kingdom. Lactose, the primary disaccharide present in breast milk, is hydrolyzed by lactase on the external surface of the intestinal brush border (Martin and Wright, 2004). The liberated glucose and galactose are then transported across the brush border membrane by the Na^+/glu co-transporter (SGLT1) and accumulate within the enterocyte. SGLT1 is responsible for the tight coupling of two Na^+ ions and one sugar molecule across the membrane (Martin and Wright, 2004; Linquist and Meeuwisse, 1962; Lee et al., 2009). CGGM is caused by a defect in the intestinal SGLT1 transporter due to mutation in the SLC5A1 gene (182380) which is located on chromosomal 22q13.1 (Martin and Wright, 2004). Lactose found in breast milk is hydrolyzed normally, but absorption of glucose and galactose is absent or reduced, leading to osmotic diarrhea. Undigested glucose and galactose are then delivered to the colon and fermented by colonic bacteria producing short chain fatty acids. The stools become acidic. Affected infants are usually present with diarrhea within the first few days of life with severe life threatening diarrhea with hyperosmolar dehydration and metabolic acidosis during the neonatal period (Martin and Wright, 2004; Lee et al., 2009; Al-Lawati and Vargees, 2008). As the patho-genesis of diarrhea is osmotic, the diarrhea resolves once enteral feeding is removed. The stool pH is usually <5.3 and stool reducing sugar is positive with large stool osmotic gap >40 mOsm. Our patient was having typical presentation of this disease and she improved dramatically after glucose and galactose were eliminated from enteral feeding and start her on fructose-based formula (Galactomin 19). The medium term prognosis of GGM is usually good. As the patients grow older, most can tolerate some amount of glucose with no diarrhea, the unabsorbed glucose being fermented by colonic bac-teria. The main concern for this group of patients is the compliance to these special formulae and the long term consequences of taking a high protein and fat diet (Martin and Wright, 2004). The required life-long glucose- and galactose-free diet may have significant renal and cardiovascular consequences (Lee et al., 2009).

While she is the first baby, her parents need to be counseled and to be seen in genetic clinic as soon as possible for the future pregnancies, fortunately our patient was accepted in higher center for that purpose.

Conclusion

Early onset diarrhea in patient with consaguionus parents should alert the pediatricians to think about a rare congenital causes of chronic diarrhea that can present with a life threatening condition or serious long-term morbidity like renal failure and gangrenous legs which happened to one of the patients who were mentioned earlier and published by our colleagues in King Khalid University Hospital in Riyadh.

REFERENCES

Guarino A, De Marco G (2004). Persistent diarrhea. In Walker WA, *et al* (eds). Pediatric Gastrointestinal disease: pathophysiology, diagnosis and management; 4e. BC Decker, Hamilton, Ontario.

Abad-Sinden A, Borowitz S, Meyers R, Sutphen J (1997). Nutrition management of congenital glucose-galactose malabsorption: a case study. J. Am. Diet. Assoc. 97:1417–1421.

Pascual JM, Wang D, Lecumberri B, Yang H, Mao X, Yang R (2004). GLUT1 deficiency and other glucose transporter diseases. Eur. J. Endocrinol. 150:627-633.

Lebenthal E, Garti R, Mathoth Y, Cohen BE, Katzenelson D (1971). Glucose-galactose malabsorption in an Oriental-Iraqui Jewish Family. J. Pediatr. 78:844-850.

Abdullah AM, el-Mouzan MI, el Shiekh OK, al Mazyad A (1996). Congenital Glucose Galactose malabsorption in Arab children. J. pediatr. gastroenterol. nutr. 23:561-563.

Assiri A, Saeed A, Alnimri A, Ahmad S, Saeed E, Jameel S (2013). Five Arab children with glucose-galactose malabsorption. Paediatr Int. Child. Health 33(2):108-10.

Martin MG, Wright EM (2004). Congenital intestinal transport defects. In: Walker WA, et al (eds). Pediatric Gastrointestinal disease: pathophysiology, diagnosis and management; 4thed BC Decker, Hamilton, Ontario.

Linquist B, Meeuwisse GW (1962). Chronic diarrhea caused by monosaccharide malabsorption. Acta Paediatr. 51:674-85.

Lee WS, Tay CG, Nazrul N, Paed M, Chai PF (2009). A Case of Neonatal Diarrhoea Caused by Congenital Glucose-galactose Malabsorption. Med. J. Malaysia 64(1):83-5.

Tawfiq A-L, Thomas V (2008). Glucose Galactose Malabsorption complicated with Rickets and Nephrogenic Diabetes Insipidus. Oman Med. J. 23(3):197–198.

Full Length Research Paper

Biochemical, haematological and histological effects following Escravos crude oil ingestion by Chinchilla rabbits

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The negative consequences of crude oil exploration and exploitation on the health status of exposed individuals cannot be over emphasized, regardless of its financial benefits. Besides, consumption of this crude oil by the rural populace living in oil rich regions as traditional medicine for illnesses have raised local and international questions as to its safety. The aim of this study was to investigate the pathological effects related to Escravos crude oil ingestion by Chinchilla rabbits. A total of thirty Chinchilla rabbits of age twelve to fourteen weeks and weighing 1.2 to 1.45 kg was used. Crude oil was orally given at the dose of 15, 20, 25 and 30 mg/kg body weight, corresponding to groups B, C, D and E, respectively for 28 days, while group A was the Control. The result showed a significant increase in the total white blood cell, monocyte, granulolytic leucocyte, platelet counts, C-reactive protein and serum creatinine ($p < 0.05$). Microscopy of the stained tissue sections showed marked deposition of collagen fibers, glomerulonephritis and atrophic glomeruli among others. There is an agreement between the biochemical, haematological and histological findings. Thus, Escravos crude oil is suggested to have a potential to cause haematoxicity and alter the architecture of the kidney.

Key words: Rabbits, creatinine, C-reactive protein, collagen fibers, Escravos crude oil, granulolytic leucocyte, kidney, microscopy, monocytes, platelets.

INTRODUCTION

Crude oil exploration is the mains stay of the Nigerian economy and constitute about 90% foreign exchange earning of the nation (Eyong et al., 2004). The over dependence on the monetary benefit of exploration and exploitation and neglect of its environmental conesquences has made the problem of crude oil pollution insurmountable. The impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration activities is an obvious problem of environmental concern (Otitoju and Onwurah, 2007; Ovuru and Ekweozor, 2004).

The largest contributor to the oil spill in total, besides corrosion of pipes and tanks, is the rupturing or leaking of production infrastructures that are described as, "very old and lack regular inspection and maintenance" (Nwilo and Badejo, 2001).

According to Dede et al. (2002), cases of misuse of this substance by individuals have been reported, as it is known to be used liberally by some of the indigenes who believe that it can repel witches when applied either topicaly, or by oral administration on afflicted individuals,

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while other countries such as Kenya, Tanzania, Zimbabwe, Ghana and Tunisia depend on crude oil for unorthodox treatment of ailments such as stomach ache, diarrhoea, respiratory distress and convulsion. The hydrocarbons in crude oil are mostly alkanes, cycloalkanes and various aromatic hydrocarbons while the other organic compounds contain nitrogen, oxygen, sulphur and trace amount of metals such as iron, nickel, copper and vanadium (Speight, 1999).

Generally, various studies on crude oil have revealed that it has serious deleterious effects on soils (Erdogan and Karaca, 2011; Jeroh et al., 2011; Mary and Dolor, 2007), plants (Baek et al., 2004; Agbogidi et al., 2007), aquatic life (Ndimele et al., 2010; Daka and Ekweozor, 2004) and even organisms such as the macrobenthic invertebrates (Arimoro and Adamu, 2008). Commonly reported effects of acute exposure to crude oil through inhalation or ingestion include: difficulty in breathing, headaches, nausea, confusion and other central nervous system effects (Akpofure et al., 2000). The aim of this study was to investigate the haematological effects and renal problem ascribed to Escravos crude oil ingestion by Chinchilla rabbits.

MATERIALS AND METHODS

Test sample

The Escravos blend crude oil (with reference number 863) used in this study was provided by Warri Refining and Petrochemical Company Effurun, Delta State. The crude oil was exposed to sunlight in shallow pans (25 cm × 25 cm × 5 cm) for 24 h at the site of the project to allow the extremely light and volatile fractions to evaporate leaving behind the stable components. This product simulates the naturally occurring condition following spillage (Neff et al., 2000).

Animals/experimental design

A total of 30 Chinchilla rabbits aged 12 to 14 weeks weighing 1.2 to 1.45 kg were obtained from the Faculty of Agriculture, Ebonyi State University Abakaliki. The animals were examined, treated for ectoparasites (using Lymectin; Hebei New Century Pharmaceutical C0. Ltd) and Bacterial infections (using Spectropan; Pharma swede-Egypt) by a veterinarian and allowed to acclimatize for two weeks at the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The rabbits were sex-matched and divided into five groups, containing 6 rabbits each (3 males and 3 females). The research plan used consist of four groups designated Group A (control), B, C, D and E. Group B to E were orally given a sub-lethal dose of 15, 20, 25 and 30 mg/kg body weight of Escravos crude oil, respectively with due consideration of their body weight (those with greater body weights have their dose divided into two; one in the morning one at night). The different doses of the liquid Escravos crude oil were measured in weight on an electronic weighing balance and given orally (oral gavage) for 28 days.

Animal treatment/sample collection

The protocol for animal handling and collection of samples in this study was approved by the Ethics Committee of the Faculty of Health

Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Nigeria. Overnight, prior to exposure, the animals (rabbits) were starved of solid food and their body weights were taken weekly and for the duration of the study to check for weight loss or gain which is associated with toxicity. The rabbits were fed vital grower pallets and water *ad libitum* for 28 days.

Organ harvest and tissue processing for light microscopy

The animals were anaesthetized using cotton wool damped in chloroform with due consideration of their body weights and sacrificed (on the 29th day morning). 5 ml of blood samples, obtained by marginal ear vein puncture, were drawn into tubes using 22 gauge sterile needles. For biochemical analyses, 3 ml of blood samples collected into plain test tubes were centrifuged (ROTOFIX 32®-HETTICH) at 3000 rpm for 10 min; the serum was collected and kept at -20°C until analysis. For the haematological investigations, 2 ml of whole blood samples were dispensed into ethylene diamino-tetra-acetic acid (EDTA) containers. The excised kidneys were blotted dry to remove traces of blood and then weighed electronically (using 210/0.1 mg digital balance ESJ-210-4) and fixed in 10% formal saline. Tissues of 3 μm thickness were stained using Haematoxylin & Eosin (H&E) and Gomori's trichrome staining techniques, and photomicrograph of the stained tissue sections was taken for documentation (Awvioro, 2002). The kidneys were processed at the University of Nigeria Teaching Hospital (UNTH), Enugu State.

Biochemical analysis and haematological investigation

Serum creatinine and urea were estimated using the Jaffe slot alkaline picrate and Urease-bertholot methods, respectively (Sood, 2009). The enzyme linked immunosorbent quantitative method was used to determine the concentration of C-reactive protein in the serum. Kits from Randox Laboratories, United Kingdom and Diagnostic Automation Inc., Calabasas were used. These biochemical analysis were done using ELISA machine (MR 96 USA) and spectrophotometer. The haematological investigations were carried out by means of automation, using the Erma Inc. Hematology Analyzer Model PCE-210. The experiment was carried out using the facilities of Reene Laboratories Onitsha and the Nnamdi Azikiwe University Teaching Hospital (NAUTH).

Statistical analyses

Mean values ± standard deviation (SD) of the sex hormones, cholesterol, body and ovary weights were taken for analysis. The data was tested for homogeneity of variance and significantly different results were established by one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) software application (version 16). The multiple comparisons were made using the Post hoc test. The accepted level of significance was set at $p < 0.05$.

RESULTS

Behavioural effect

After two days of the crude oil administration, the animals in the treated groups D and E became restless. The latter was followed by loss of appetite and decreased locomotion. They regained their appetite after the tenth

Table 1. Mean ± SD of the biochemical parameters change in body weight of animals per week (kilogram) and weight of kidney (kilograms) in the test and control groups.

Weight	Group					F-value	P-value
	A (control)	B (15 mg/kg)	C (20 mg/kg)	D (25 mg/kg)	E (30 mg/kg)		
C-reactive protein (mg/dl)	0.24±0.11	0.38±0.12	0.51±0.25	0.57±0.19	0.46±0.07	3.751	0.019
Creatinine (µmol/l)	0.33±0.08	0.33±0.06	0.36±0.08	0.48±0.09	0.38±0.06	3.946	0.015
Urea (mmol/l)	4.97±1.44	5.66±0.87	6.00±1.16	6.60±1.68	6.00±1.35	1.102	0.385
Mean change in weight (kg)	0.12±0.04	0.09±0.03	0.02±0.01	0.08±0.04	0.05±0.02	4.636	0.019
Weight of kidneys (kg)	0.081±0.009	0.092±0.011	0.103±0.010	0.108±0.011	0.103±0.06	8.109	0.001

P is significant at $p < 0.05$

Table 2. Total white cell counts and differential values in rabbits exposed to crude oil (mean ± SD).

Groups/dose	WBC ($10^3/\mu l$)	Lymphocytes ($10^3/\mu l$)	Monocytes ($10^3/\mu l$)	Granulolytic leucocytes ($10^3/\mu l$)
	Mean±SD			
A (00 mg/kg)	4.50±0.57	1.50±0.14	0.30±0.01	2.70±0.71
B (15 mg/kg)	5.20±0.10	2.23±0.31	0.87±0.21	2.90±1.42
C (20 mg/kg)	6.17±0.21	1.53±0.71	0.50±0.26	4.10±1.04
D (25 mg/kg)	7.17±0.61	2.50±0.66	0.90±0.30	3.80±0.17
E (30 mg/kg)	9.60±0.53	2.77±0.74	1.33±0.55	5.50±0.87

day, though not completely. Table 1 shows significant increase in the serum concentrations of C-reactive protein, creatinine, mean change in body weight per week and kidney weight ($p < 0.05$), with an insignificant increase in serum urea concentrations. The pair-wise comparison was made between the control group and the treated groups. The post hoc test (LSD) showed significant increase in total white blood cells (WBC), monocyte and granulocytic counts ($p < 0.05$) in a dose dependent manner, with slight increases in other haematological parameters.

DISCUSSION

Crude oil(s) extracted from different wells and locations have different chemical compositions, which may finally determine their toxicity (Neff et al., 2000). The result of this study elucidates the potency of Escravos crude oil to induce haematoxicity and renal pathology in relation to haematological parameters, CRP, creatinine, urea and histology.

According to Jacob and Al-Muzaini (1995), petroleum pollution could range from diffused chronic exposure to considerably large single doses. These sub-lethal concentrations may not necessarily lead to outright mortality but may have significant effects which can lead to physiological stress and dysfunctions in animals (Omoregie, 1998). The high total WBC counts observed in this study (Tables 2 and 4) is in accordance with the report of Eyong et al. (2004) who orally administered

the Bonny Light crude oil to rabbits but discordant with the reports of Ovuru and Ekweozor (2004) who administered Nigerian Agip crude oil to rats in doses of 0.05, 0.10, 0.15 and 0.20%. The increase in WBC counts could be adduced to the normal physiologic response following perception of a foreign attack by the body defence mechanisms. The result also showed the granulocytic leucocyte count (neutrophils and eosinophils) significantly increased linearly with increasing concentration of crude oil (Tables 2 and 4) which is in accordance with the reports of Ovuru and Ekweozor (2004). This increase is an indication of stress imposed by crude oil fraction in the diets and supported by Selye (1963), finding that a stress stimulus elicits a defence response. Leucocytosis is found in several conditions which include

Table 3. Total red cell counts, platelets and dependable factors in rabbits exposed to crude oil.

Groups/dose	RBC (10^3/µl)	Platelets (10^6/µl)	Haemoglobin (g/dl)	PCV (%)
A (00 mg/kg)	6.11±0.49	178.50±33.23	10.50 ±0.99	32.05±3.46
B (15 mg/kg)	6.03±0.48	288.67±44.97	10.50±0.43	30.97±2.38
C (20 mg/kg)	6.31±0.60	201.00±49.15	10.53±0.84	32.10±2.75
D (25 mg/kg)	6.42±0.30	235.33±30.37	10.97±0.83	32.20±3.74
E (30 mg/kg)	6.24±1.39	318.00±74.54	10.53±2.86	31.33±5.91

Table 4. The pairwise comparison (Post hoc test) of mean ± SD of the haematological parameters.

Parameter	WBC	Lymphoctes	Monocytes	Granulocytes	Rbc	Hgb	PCV	Platelets
A (00 mg/kg)	-	-	-	-	-	-	-	-
B (15 mg/kg)	0.115	0.158	0.097	0.824	0.913	1.000	0.767	0.041
C (20 mg/kg)	0.002	0.952	0.530	0.144	0.782	0.981	0.989	0.637
D (25 mg/kg)	0.000	0.098	0.082	0.240	0.674	0.742	0.967	0.249
E (30 mg/kg)	0.000	0.044	0.008	0.011	0.859	0.981	0.845	0.014
F-value	56.02	2.555	3.694	3.733	0.114	0.052	0.058	3.572
P-value	0.000	0.112	0.048	0.047	0.974	0.994	3.572	0.052

P is significant at $p < 0.05$.

include: inflammation, tissue necrosis and polycythaemia vera (Hoffbrand et al., 2001). Also observed is a significant increase in the monocyte count (Table 2), which suggests that the crude oil increased the susceptibility of the rabbits to bacterial infection- resulting in monocytosis. This is in contrast to the works of Ovuru and Ekweozor (2004) who reported a decrease in the monocyte count after administering 0.05, 0.10, 0.15 and 0.20% of Nigerian Agip oil. The main function of platelets is to form mechanical plugs during normal haemostatic response to vascular injury (Hoffbrand et al., 2001), which could be the which could be the reason for the observed high platelet Count (Table 3).

Increase in haemoglobin (Hb), red blood cell (RBC) count, packed cell volume (PCV), and platelet count are found in myeloproliferative disorders such as polycythaemia vera (Hoffbrand et al., 2001), this could be related to high RBC count, lymphocyte count, and Hb values observed in this study (Table 3 and 4). Furthermore, Hb concentrations are reported to be moderately increased in haemolytic anaemia and any condition associated with rapid intrascular haemolysis and haemoglobinuria (Bolarin, 1997).

C-reactive protein (CRP) is an acute phase protein synthesized by the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl (Kushner and Rzewnicki 1994; Macy et al., 1997). Its physiological roles are numerous and varied, but with several functions similar to those of immunoglobulins, CRP appears to function in host defense (Schultz and Arnold, 1990). As elevated CRP values are always associated with pathological changes, the CRP assay provides useful information for the diagnosis, therapy and monitoring of inflammatory processes and associated disease (Shine et al., 1981; Dixon, 1984; Hind and Pepys, 1984; Kushner, 1991). The result of this study showed high CRP concentration in the treated groups when compared with the control group. The Inflammatory process marked by lymphocytic infiltration and hyperchromatic fibroblast evident in the stained tissue section (Figure 1 to 6) could be related to the high CRP and granulolytic leucocytes.

Renal azotaemia occurs when urea is retained primarily due to impaired glomerular filtration which results in acute or chronic renal disease. The acute state may be due to glomerulonephritis, nephrotoxic drugs, or renal cortical necrosis (Ochei and Kolhatkar, 2000). The high serum concentrations of creatinine and urea observed in the treated groups when compared with the control group are in agreement with the findings of Azeez et al. (2013) who exposed rats to petroleum hydrocarbon.

In diseases such as necrotizing, crescentric glomero-nephritis where there is disruption of Bowman capsule, fibroblasts can migrate from the interstitium into glomeruli and produce interstitial collagens (Guillermo and Elba, 2010). The latter was evident in the stained tissue sections of the treated groups in this study (Figures 4 and 6). A replacement of mesangium with eosinophilic amorphous material is an indication of light chain amyloidosis (Guillermo and Elba, 2010). This eosinophilic material was observed in the stained sections of the treated groups (Figure 5).
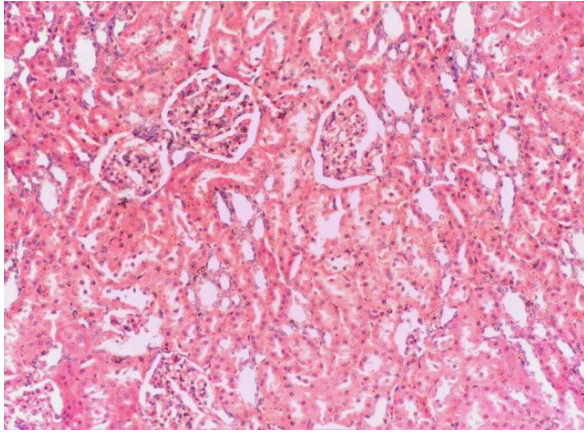
Figure 1. Group A: Photomicrograph of kidney section with no obvious pathology.
Stained by H&E technique. ×200.
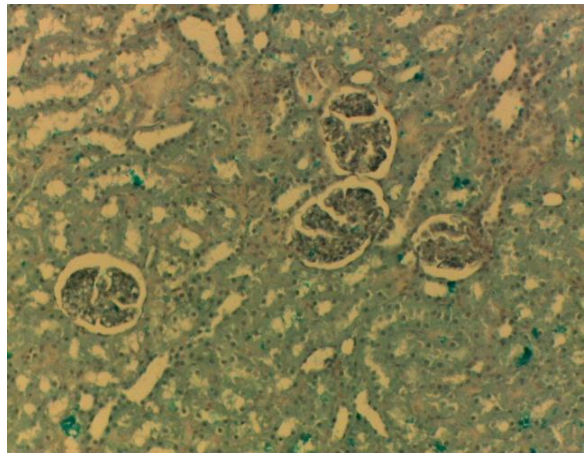
Figure 2. Group A: Photomicrograph of a kidney section with no obvious pathology.
Gomori's Trichrome technique. ×200.
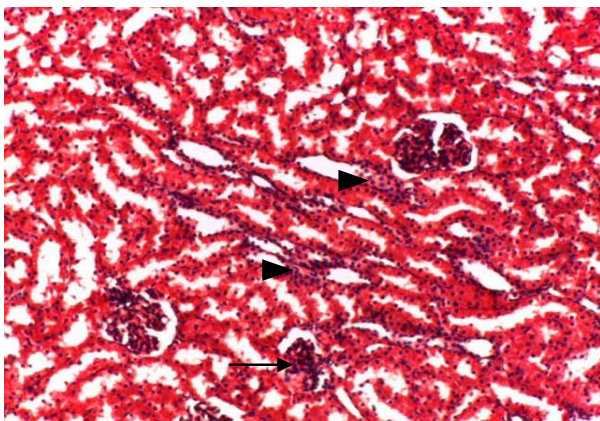
Figure 3. Group B (15 mg/kg): Photomicrograph of the kidney with marked lymphocytic infiltration (glomerulo-nepheritis marked by arrow heads), marked cellularity within the tuft (migrated fibroblasts). The tubules are slightly enlarged and with a shrunken glomerulus (arrow).
Stained by H&E technique. ×200.
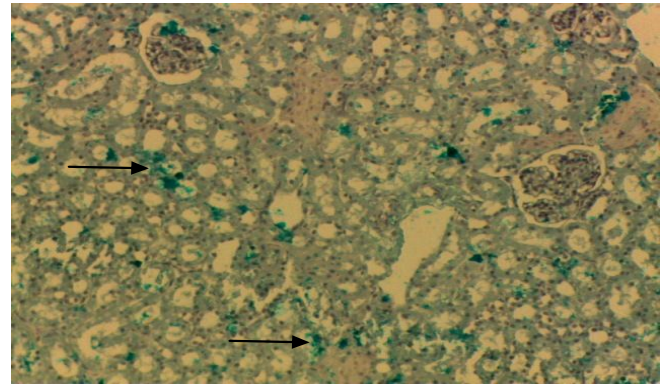
Figure 4. Group C (20 mg/kg): Photomicrograph of a section of the kidney showing enlarged renal tubules, marked cellularity and mild deposition of collagen fibres (green marked by arrows).
Stained by Gomori's trichrome technique. ×200.
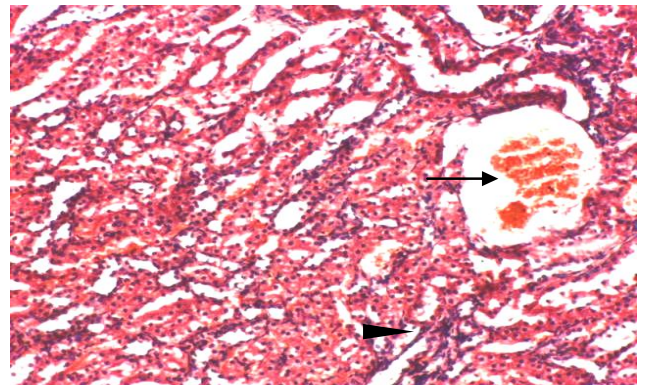
Figure 5. Group D (25 mg/kg): Photomicrograph of the kidney with some congested tubules with hyperchromatic fibroblasts and fibrosis (arrow heads). There is an enlarged cystic space with eosinophilic material (a possible indication of light chain associated amyloidosis). Some of the tubules are enlarged with single layer of epithelium.
Stained by H&E technique. ×200.
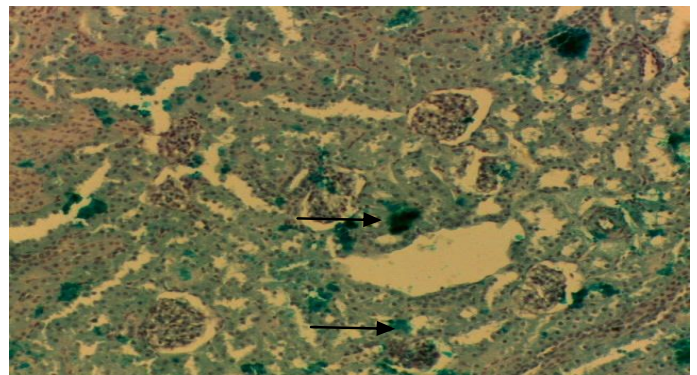
Figure 6. Group D (25 mg/kg): Photomicrograph of a section of the kidney with enlarged Bowman's capsule, cystic space, shrunken glomeruli, lymphocytic and fibroblastic infiltration mar-ked deposition of collagen fibers (green marked by arrow) and stromal erosion. Stained by Gomori's trichrome technique. ×200.
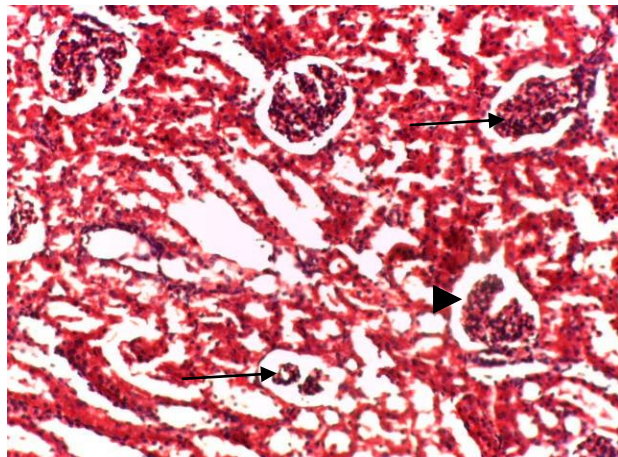
Figure 7. Group E (30 mg/kg): Photomicrograph of the kidney with multicystic stromal tissue with few identifiable tubules, Slightly shrunken (arrow) and atrophic glomeruli (arrow), and enlarged Bowman's capsule (arrow head). Stained by H&E technique. ×200.

Conclusion

The findings of this study suggest that Escravos crude oil, even in low concentration, has the potential to affect haematological, biochemical parameters and can cause renal diseases following exposure.

ACKNOWLEDGEMENTS

REFERENCES

Azeez OM, Akihigbe RE, Anigbogu CN (2013). Oxidative status in rat kidney exposed to petroleum hydrocarbons. J. Nat. Sci. Biol. Med. 4(1):149-54.

Agbogidi OM, Eruotor PG, Akparobi SO (2007). Effects of crude oil levels on the growth of maize (*Zea mays* L.) Am. J. Food Technol. 2:529-535.

Akpofure EA, Efere ML, Ayawei P (2000). The adverse effect of crude oil spills in the Niger Delta; Urhobo Historical Soc. J. 10:10-14.

Arimoro FO, Adamu KM (2008). Toxicological effects of water soluble fraction of crude oil on macrobenthic invertebrates: Chironomus and mosquito larvae. Res. J. Environ. Toxicol. 2:23-26.

Awvioro OG (2002). Histochemistry and Tissue Pathology Principles and Techniques, First Edition, ISBN 978 – 35627 – 9. pp. 154 – 162.

Baek KH, Kim HS, Oh HM, Yoon BD, Kim J, Lee IS (2004). Effects of crude oil components and bioremediation on plant growth. J. Environ. Sci. Health Part A. 39:2465-2472.

Bolarin DM (1997). A Handbook on Clinical Chemistry. Nelson Publishers Limited, Illupeju, Lagos, Nigeria. P 412.

Daka ER, Ekweozor IKE (2004). Effect of size on acute toxicity of crude oil to the mangrove Oyster *Carasosstrea gasar*. J. Appl. Sci. Environ. Manage. 8:19-22.

Dede EB, Igboh NM, Ayalogu OA (2002). Chronic study of crude petroleum bonny light, kerosene, and gasoline using haematological parameters. J. Appl. Sci. Environ. Manage. 6(1):60-66.

Dixon JS (1984). C-reactive protein in the serial assessment of disease activity in rheumatoid arthritis. Scand. J. Rheum. 13:39-44

Erdogan EE, Karaca A (2011). Bioremediation of crude oil polluted soils. As. J. Biotechnol. 206-213

Eyong EU, Umoh IB, Ebong PE, Eteng MV, Anatai AB, Akpa AO (2004). Haematoxic Effects Following Ingestion of Nigerian Crude Oil and Crude Oil Polluted Shellfish by rats. Niger. J. physiol. studies. 19(1-2):1–6.

Guillermo AH, Elba AT (2010). Renal diseases with organized deposits; An algorithmic Approach to classification and clinicopathologic Diagnosis. Arch. Pathol. Lab. Med. 134:512-531

Hind CRH, Pepys MB (1984). The role of C-reactive protein measurement in clinical practice. Int. Med. 5:112-151

Hoffbrand AV, Pettit JE, Moss PAH (2001). Essentials of haematology: The white cell, Myeloproliferative disorders. 4 ed. ISBN 0-63205-153-1, Blackwell Science ltd. pp.120-229.

Jacob PG, Al-Muzaini S (1995). Marine plants of the Arabian Gulf and effects of oil pollution. Mahasagar 23:83-101.

Jeroh E, Tonukari NJ, Anigboro A (2011). Glucose level and amylase activity in crude oil contaminated soil bioremediated with poultry Manure and sawdust. As. J. Biol. Sci. 4:369-374

Kushner I (1991). C-reactive protein in rheumatology. Arthritis Rheum. 34:1065-1068

Kushner I, Rzewnicki DL (1994). The acute phase response: General aspects. Bailliere's Clin. Rheumatol. **8**:513-530

Macy EM, Hayes TE, Tracy RP (1997). Variability in the measurement of C-reactive protein in healthy subjects: implications for reference interval and epidemiological applications. Clin. Chem. 43(1):52-58

Mary AO, Dolor DE (2007). An assessment of the growth of irvinga gobenensis (Aubry-lecente Ex O'Rorte) bail seedlings as influenced by crude oil contaminated soil. Asian J. plant Sci. 6:1287-1292

Ndimele PE, Jenyo-Oni A, Jibuike CC (2010). Comparative toxicity of crude oil, dispersant and crude oil-plus-dispersant to *Tilapia guineensis*. Res. J. Environ. Toxicol. 4:13-22

Neff JM, Ostazeski S, Gardner W, Steiskal I (2000). Effects of weathering on the toxicity of three offshore Australian crude oils and diesel fuel to marine animals. Environ. Toxicol. Chem. 19: 809 - 820.

Nwilo PC, Badejo OT (2001). Impact of Oil Spills along the Nigeria Coast. The Association for Environmental health Sciences.

Ochei JO, Kolhatkar AA (2000). Medical Laboratory Science, Theory and Practice; renal function test and urine analysis. Tata McGraw-Hill Publishing Company Ltd. pp.116-122.

Omoregie E (1998). Changes in the haematology of the Nile tilapia (*Oreochromis niloticus* Trewavas) under the effects of crude oil. Acta Hydrobiol. 40:287-292.

Otitoju O, Onwurah INE (2007). Preliminary investigation into the possible endocrine disrupting activity of Bonny light crude oil contaminated diet on Wistar rats. Biokemistri J. 19(2):23-28.

Ovuru SS, Ekweozor IKE (2004). Haematological changes associated with crude oil ingestion in experimental rabbits. Afr. J. Biotechnol. 3(6):346-348.

Selye H (1963). Stress and the adaptation syndrome. In: Encyclopaedia of medicine, surgery and specialities. E.A. Davis Co. New York. 13:365-366.

Schultz DR, Arnold PI (1990). "Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, glycoprotein, and fibrinogen." Seminars in Arthritis Rheum. 20:129-147.

Shine B, de Beer FC, Pepys MB (1981). Solid phase radiommunoassays for human C-reactive protein. J. Lab. Clin. Chimica Acta. 117:13-23.

Sood R (2009). Medical Laboratory Technology method and interpretations, Sixth Edition. Jaypee Brothers Medical Publishers (P) LTD, ISBN 9788184484496; pp.1413–1431.

Speight JG (1999). The Chemistry and Technology of Petroleum, 4th eds. CRC Press United Kingdom. pp.16-38.

Full Length Research Paper

Evaluation of antibacterial profile of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from hospitals in Imo state, Nigeria

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Awareness of the threat of methicillin resistant *Staphylococcus aureus* (MRSA) is growing. Oxacillin and methicillin are penicillinase-stable penicillins, and strains that are oxacillin and methicillinn resistant are historically termed MRSA. To determine the existence of MRSA strains patients attending treatments in hospitals in Imo state, 200 clinical specimens were examined using conventional method such as culture and sensitivity. The specimens include urogenital swabs, nasal swabs, wound swabs, pus and blood. Out of the 200 samples collected, 23.68% showed resistance to oxacillin, 25.00% of the isolates from Owerri Zone were MRSA, while 23.81% of isolates from Okigwe Zone yielded MRSA, and 20.00% from Orlu Zone yielded MRSA. The mean for zones is 25.33 ± 13.05. Children under the age of 10 have the highest incidence of (57.14%) of *S. aureus* isolates resistant to oxacillin, followed by the elderly people of age group 51 to 60 years (27.27%). The young adults between the ages of 21 to 30 have the least incidence (10.00%) and the mean for age groups is 43.66 ± 6.26. The mean for clinical specimens is 15.20 ± 22.16. Wound specimens produced the highest incidence (40.00%) among clinical specimens followed by pus (33.33%) and blood has the least occurrence (00.00%). Males produced the highest incidence between the sexes (26.67%) and women (21.74%), and the mean for sexes is 38.00 ± 11.31. This shows that MRSA exists in hospitals in Imo state and considering the danger, it portends to healthcare setting, and efforts are needed to contain its spread.

Key words: Methicillin, antibiotics, zones, sex, children, specimen, infections.

INTRODUCTION

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an anti-biotic. Genes can be transferred between bacteria in a horizontal fashion by conjugation, transduction, or transformation. Thus a gene for antibiotic resistance which had evolved via natural selection may be shared.

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Evolutionary stress such as exposure to antibiotics was then selected for the antibiotic resistant trait. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. If a bacterium carries several resistant genes it is called multi-resistant or informally, a superbug or super bacterium (Noskin et al., 2005).

The primary cause of antibiotic resistance is genetic mutation in bacteria. The prevalence of antibiotic resistant bacteria is a result of antibiotics use both within medicine and veterinary medicine. The greater the duration of exposure, the greater the risk of the development of resistance irrespective of the severity of the need for antibiotics. As resistance towards antibiotic becomes more common, a greater need for alternative treatments arises. However, despite a push for new antibiotic therapies, there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem.

During the late 1950s and early 1960s, *Staphylococcus aureus* caused considerable morbidity and mortality as a nosocomial, or hospital acquired, pathogen. Since then, penicillinase-resistant, semi synthetic penicillins have proved to be successful antimicrobial agents in the treatment of Staphylococcal infections. Unfortunately, methicillin-resistant *S. aureus* (MRSA) strains have recently emerged as major nosocomial problem. One way in which Staphylococci become resistant is through acquisition of a chromosomal gene (*mecA*) that encodes an alternate target protein which is not inactivated by methicillin (Proctor, 2008).

S. aureus is a non-motile gram-positive cocci that appears in clusters. It is found worldwide and is a leading cause of infectious disease. It can normally only transiently colonize the outside and entry portals of the human body (skins, ears, eyes, nasal passages etc.) but it is estimated that 20% of humans are carriers (asymptomatic permanent colonizatiion) (Kluytmans et al., 1997). However, even transient colonization can lead to infection if the conditions are right, such as a breach in the protective layer of epithelial cells, or a compromised immune system. The ability to cause disease is via two mechanisms, namely; toxin production and proliferation of the organism, which causes tissue destruction. Most infections remain localized at entry portals and are usually self-limiting and non-life threatening. Much less frequently, more serious infections may occur when the organism is able to invade deeper into the body (osteomyelitis, septicemia, pneumonia etc). These deeper infections may be extremely serious and even fatal because infections of *S. aureus* occur at a higher rate than that of many bacteria. The costs that are incurred for hospitalization and treatment can be tremendous.

In just one year, 1995 in New York City, it was estimated that there were at last 13,550 cases of *S.aureus*

infections resulting in an estimated cost of about $435.5 million (Rubin et al., 1999). In a study that was published in 2007 with data from the year 2003, it was found that nearly 390,000 people in the US were hospitalized with *S. aureus* infections, with the average hospital stay for this type of infection costing $37,251. The total cost of *S. aureus* infections in the US for 2003 was $14.5 million (Noskin et al., 2007).

Before the advent of antibiotics, the mortality rate from *S. aureus* infections was near 80%. When pencillin therapy was introduced in the 1940s, it seemed that *S. aureus* (along with many other bacteria) infections could now be easily treated. However, within a short amount of time penicillin – resistant *S. aureus* had appeared, with approximately 80% of *S. aureus* now being penicillin resistant (Deurenberg et al., 2007). This led to the discovery and use of other antimicrobials such as methicillin in the 1960s.

Once again, the organism seemed to be under control. It was not long however before methicillin – resistant *S. aureus* (MRSA) appeared, and strain that were sensitive to methicillin became known as methicillin-susceptible *S. aureus* (MSSA) (Chambers, 2001). Then clinicians turned to the use of vancomycin for serious MRSA infections, and recently we have seen that MRSA strains are evolving that are able to overcome vancomycin. A few vancomycin-intermediate resistant *S. aureus* (VISA) have been isolated (Smith et al., 1999).

Oxacillin is a parental second generation penicillin antibiotic that is used to treat moderate-to-severe penicillinase-resistant Staphylococci infections. It was approved for use in United State in 1989 and is still in common use (CDC, 2010). Oxacillin and methicillin are penicillinase-stable penicillins and strains that are oxacillin and methicillins resistant and are historically termed methicillin-resistant *S. aureus* (MRSA) (Clinical and Laboratory Standard Institute, US, 2007). Because methicillin is no longer commercially available in the United State, oxacillin maintains its activity during storage better than methicillin and is more likely to detect hetero-resistant strains. Hence oxacillin is tested instead of methicillin (CLSI, 2007).

The isolate tested with oxacillin is called MRSA instead of ORSA because when resistance was first described in 1961, methicillin was used to test and treat infections caused by *S. aureus.* However, oxacillin which is in the same class of drugs as methicillin was chosen as the agent of choice for testing *Staphylococcus* in the early 1990s. The acronym MRSA is still used by many to describe these isolates because of its historic role (Bannerman, 2003).

MRSA was discovered in 1961 in the United Kingdom. It made its first major appearance in the United States in 1981 among intravenous drug users. MRSA is often referred to in the process as a SUPERBUG. The number

of MRSA infection in the United States has been increasing significantly. A 2007 report in Emerging Infectious Disease, a publication of the Centres for Disease control and Prevention (CDC 2007), estimated the number of MRSA infection in hospitals doubled nationwide, from approximately 127,000 in 1999 to 278,000 in 2005, while at the same time annual deaths increased from 11,000 to more than 17,000 (Klein et al., 2007) and estimated that MRSA would have been responsible for 94,360 serious infections and associated with 18,560 hospital stay-related deaths in the United State in 2005 (Klevens et al., 2007; CDC, 2007). These figures suggest that MRSA infections are responsible for more death in the US each year than AIDS (Stein, 2007). Kleven (2007) also suggested that in the incidence of invasive infections caused by USA, 300 remains more in rural than in the urban centers.

Though MRSA since its emergence has become a major cause of illness and death in the healthcare set-tings, no serious previous attempt has been made to stu-dy its profile in the hospitals situated in Imo State. Before now Imo inhabitants have the belief that patients only go to hospitals to be treated and not to contact infections. This research shall therefore assess the prevalence of this MRSA amongst the hospitals in Imo State, determine the gender, zone, age-group and clinical specimen that yielded most of the MRSA and proffer solution to prevent epidemics due to hospital acquired MRSA.

MATERIALS AND METHODS

Discription of the study area

The study area is Imo State, located at the South Eastern part of Nigeria. Lies within latitudes 4° 45' N and 7° 15' N and longitude 6° 50' E and 7° 25' E. It is bounded in the South by Rivers State, in the North and East by Abia State and in the West by Anambra State. It has many primary, secondary and tertiary health institutions among which are the health centres at the community levels, the General Hospitals under the State Hospital Management Board (HMB) at every Local Government Area and health institutions like Federal Medical Centre Owerri and Imo State University Teaching Hospital Orlu which serve as referral centres for Imo State and neighbouring States.

Sample and sampling technique

A total of two hundred (200) samples were collected from patients who have been admitted for about 2 to 4 days from the selected hospitals between the month of October, 2010 and August, 2011, using random sampling method on both manitol salt agar and blood agar medium. All specimens were cultured within 3 h of collection. The selected hospitals include: General Hospitals, Okigwe in Okigwe zone, General Hospital Uguta in Orlu zone and General Hospital Owerri in Owerri zone, to represent the three geographical zones in Imo State. Samples were obtained from the following clinical specimens: Urogenital specimens (including urine, urethral

swabs, vaginal swabs, and semen), nasal swabs, wound swabs pus and blood.

Ethical consideration

Permission was sought from Imo State Ministry of Health under which the Health Management Board operates. Letter of authority from this ministry was presented to each of these hospitals before samples were allowed to be collected from in-patients.

Innoculation, isolation and identification of organisms

The specimens were inoculated on manitol salt agar and blood agar plates using the streak technique to obtain discrete colonies while blood samples were first inoculated into brain heart infusion broth, incubated for 24 h before being transferred to the solid media. This transfer was repeated every day for 7 days, before it was considered "no bacterial growth". The plates were incubated at 37 and 30°C for manitol salt and blood agar, respectively for 24 h under aerobic conditions. The culture plates were examined recording to appearance, size, colour and morphology of colonies. Gram stain reaction, catalase and coagulase test were carried out on the isolates. Isolates that were gram positive cocci, catalase positive and coagulated human plasma were considered as *S. aureus* (Chigbu and Ezeronye, 2003; Uabi-Egbeni, 2003).

Susceptibility of isolates to oxacillin

Antimicrobial susceptibility of isolates to oxacillin was carried out on *S. aureus* isolates using the paper disc diffusion technique. A. 0.06 ml of overnight culture of the test organism (Mc Ferland standard 0.5) was seeded on Mueller Hinton agar plate. This was spread over the entire surface of the agar plate using a sterile glass spreader and allowed to dry for about 15 to 30 min. The 5 µg oxacillin antibiotic disc (oxoid) was then placed on the agar plates using sterile forceps. The inhibition zone diameter (IZD) was measured and recorded in millimetres.

Minimum inhibitory concentration (MIC)

To confirm the resistivity of the *S. aureus* isolates to oxacillin, minimum inhibitory concentration (MIC) of oxacillin for the isolates by agar dilution method was determined. Antibiotic stock solution (10,000 mg/l) of oxacillin powder obtained from oxoid was prepared according to manufacturers instruction. A two-fold serial (double) dilution for series of the antimicrobial agent was prepared in 30 ml containers, including a drug free control. 19 ml of molten Mueller-Hinton agar at 50°C was added to each of the containers, mixed thoroughly and poured into pre-labelled sterile petri dishes on a level surface. It was allowed to solidify at room temperature and was dried in the incubator to remove moisture. Then, from a 10^4 cfu density (Mc Farland standard) of *S. aureus* solution already prepared, the plates including the control were inoculated using a standard wire loop. The inoculum spots were allowed to dry at room temperature before inverting the plates for incubation at 30°C for 18 h under aerobic condition. The trailing end point was investigated by sub-culturing and re-testing to confirm β-lactamase production.

Statistical analysis

The parameters estimated are the proportion resistant to oxacillin,

Table 1. Distribution of respondents who yielded *Staphylococcus aureus* according to Zone.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	n i2/f (pi)	% pi
Orlu	12	3	15	0.2000	20.00
Okigwe	16	5	21	0.2381	23.81
Owerri	30	10	40	0.2500	25.00

Mean (X) = 25.33 ± 13.05. Std. Deviation (S) = 13.051.

Table 2. Distribution of respondents who yielded *Staphylococcus aureus* according to age group.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	Mid point	fx	Fx^2	n i2/f (pi)	% pi
1-10	3	4	7	5.5	38.5	211.75	0.5714	57.14
11-20	6	1	7	15.5	108.5	1681.75	0.1429	14.29
21-30	9	1	10	25.5	255.0	6502.50	0.1000	10.00
31-40	5	1	6	35.5	213.0	7561.50	0.1667	16.67
41-50	8	2	10	45.5	455.0	20702.50	0.2000	20.00
51-60	8	3	11	55.5	610.5	33882.75	0.2727	27.27
61-70	19	6	25	65.5	1637.5	107,256.25	0.2400	24.00
Total	-	-	76	-	3318.0	177,799.00	-	-

Mean (X) = 443.66 ± 6.26. Std. Deviation (S) = 6.263

Table 3. Distribution of respondents who yielded *Staphylococcus aureus* according to clinical specimen.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	n i2/f (pi)	% pi
Urogenital	41	13	54	0.2407	24.07
Nasal	11	2	13	0.1538	15.38
Wound	3	2	5	0.4000	40.00
Pus	2	1	3	0.3333	33.33
Blood	1	0	1	0.0000	00.00

Mean (X) = 15.20 ± 22.16. Std. Deviation (S) = 22.163

the mean and the standard deviation of both resistivity and sensitivity from the mean. The methods of analysis are: (1) Chi – square test statistic for contingency; (2) Pair wise comparison was used to compare the proportions.

RESULTS

The distribution of samples among the three geographical zones of Orlu, Okigwe and Owerri, respectively showed that Owerri Zone had the highest incidence (25.00%) of *S. aureus* isolates resistant to oxacillin. This was followed by Okigwe Zone (23.81%) and lastly Orlu Zone (20.00%) as shown in Table 1. Table 2 shows the incidence of MRSA among different age groups. It was observed that children under the age of 10 had the highest incidence (57.14%) of *S. aureus* isolates resistant to oxacillin,

followed by the elderly of 51 to 60 years of age (27.27%). The young adults between the ages of 21 to 30 has the least incidence (10.00%). The distribution among different clinical specimens collected as shown in Table 3 indicated that wound specimen has the highest incidence (40.00%) of *S. aureus* isolates resistant to oxacillin, followed by pus specimen (33.33%). Blood has the least occurrence (00.00%). And Table 4 shows the incidence of MRSA between sexes. It was observed that males were more infected with incidence rate (26.67%) of *S. aureus* isolates resistant to oxacillin.

The mean are as follows; zones = 25.33 ± 13.05, age groups = 43.66 ± 6.26, clinical specimens = 15.20 ± 22.16 and sexes = 38.00 ± 11.31, and the standard deviations (S): zone = 13.015, age group = 6.263, clinical specimen = 22.163 and sex = 11.314.

Table 4. Distribution of respondents who yielded *Staphylococcus aureus* according to sex.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	n i2/f (pi)	% pi
Male	22	8	30	0.2667	26.67
Female	36	10	46	0.2174	23.68
All	58	18	76	0.2368	23.68

Mean (X) = 38.00 ± 11.31. Std. Deviation (S) = 11.314.

DISCUSSION

This study was done primarily to determine the level of resistant of *S. aureus* species isolated from hospitals in Imo State. It is also aimed at demonstrating the frequency and pattern of distribution among zones, specimen, gender and ages in the State. Much has been written in recent years about the methicillin resistance *S. aureus* but not much work has been done on that in this part of Nigeria.

All together, 200 individuals were screened in this study. These include 100 males and 100 females from the three geographical zones of the State and of different age groups. Also different clinical specimens were used which include; nasal, urogenital, wound, pus and blood. According to the zones of location, the percentage of *S. aureus* growth that is resistant to oxacillin is least in Orlu (with 20%) and highest in Owerri (with 25 %). The result of this therefore is in contrast with (Klevens et al., 2007) who suggested that in the incidence of invasive infections caused by USA, 300 remains more in rural areas than in the Urban center. This contradiction may be because of the central nature of Owerri which made it possible for people to be operating from every part of the State to the Capital hence are having access to hospital services at urban area even when they are living in the rural areas.

Percentage of *S. aureus* growth resistant to oxacillin decreased from about 57.14% among respondents aged 1 to 10 years to about 10.0% among those aged 21 to 30 years and thereafter, increased to about 27.27% among those aged 51 to 60 years of age. This is in line with a 2007 study by the "Archives of disease in children including hospital nurseries". This study incriminated MRSA as also becoming a problem in pediatric settings. The U curve incidence among the ages could be attributed to the low immunity of the children and the elderly. While the children have not built up enough immunity to fight incoming infections, the elderlys' immune systems may have been affected by disease like diabetes, cancer, ashma or, the many have been transplant recipients. This is in agreement with Reuters (2009) who included these categories of people among the risk population for MRSA. It is also believed that the young children and the elderly are the ages that visit

hospitals more regularly for admission. Hence the high incidence found among their age groups can be understood as a result of their frequent admissions in hospitals.

By their sexes, the percentage of *S. aureus* resistant to oxacillin is slightly higher for males (with about 26.67%) than for females (with about 21.74%). This relatively high rate in male cannot be easily explained except that the reign of motor bikes in the State made it possible for males to be visiting hospitals more frequently than the females. This is as a result of frequent accident that are occurring among the bike users which males are the major culprits.

According to the clinical specimen, *S. aureus* growth from wound showed the greatest resistance to oxacillin (with about 40%) while growth from blood and nasal specimen showed the least resistance (with about 00.00 and 18.38%, respectively). The observed high incidence rate in wound specimens can be attributed to its exposure, which makes it more possible for infections to be attracted to it. It can also be attributed to the use of antibiotic in dressing the wounds which makes it possible also for the infections to be resistant to other antibiotics. This work agreed with the findings of a previous work by Chambers (1997) who observed that the usual source of staphylococci in a ward is from septic wounds, particularly several fistulate and colostomies. If dressings from such wound are improperly handled, they could contaminate the ward, air, dust and hands of nurses and then be transferred to others. Even though nasal specimens yielded the highest incidence of *S. aureus* growth, it did not produce the highest incidence of *S. aureus* resistance to oxacillin. This can be attributed to *S. aureus* being a normal flora of the nasal passage. The zero value associated with the blood specimen may be more due to the number of cases than actual resistance. Again blood is expected to be sterile. These specific values of percentage of growth of *S. aureus* resistance to oxacillin differ greatly from the overall value (23.68%). This calls for further investigation on the degree of variation among categories of the different characteristics. This study has several limitations, among which is that considerations were not made of those who have acquired the infections before their admissions to

the hospitals, since the MRSA can also be acquired at community level. Also, this work did not involve typing of the invasive MRSA isolates.

Another is that *S. aureus* isolates resistant to other antibiotics were not considered so as to know if they are more multi drug resistance rather than oxacillin alone. Based on these limitations, some might argue that MRSA is not acquired from the hospitals in Imo State. However, MRSA certainly has become a hot topic in Nigeria, Imo State not excluded. It has been isolated at the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeriia (Taiwo et al., 2004).

CONCLUSION AND RECOMMENDATIONS

It has been confirmed that MRSA has become a major cause of illness and death in the society. Also, this research has proved that this MRSA exists in hospitals in Imo State, that children and elderly are more vulnerable. This work also proved that the choice of methicillin in treatment of *S. aureus* infection is no more fashionable and that even the use of varcomycin is becoming obsolete. We therefore recommend that a surveillance system be established by the State Government to monitor the number and incidence of infections, that there should be a monitoring body for changes in their antimicrobial susceptibility. Also, our hospital must screen the patients upon admission to prevent the cohabitation of MRSA carriers with non-carriers. Finally, education of the hospital staff as well as the patients is very vital to enable each to maintain the measures required to prevent the spread of the infections

REFERENCES

Bannerman TL (2003). *Staphylococcus, Micococcus and other Catalase – positive cocci that grow aerobically.* In P.R. Murray, E.J. Bron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken (edition) Manual of clinical microbiology 8th edition. ASM Press, Washington D.C.

Center for Disease Control (2007). Guidelines for Infection Control in Healthcare Personnel. *Centers for Disease control and Prevention* **12**:30-36.

Centers for Disease Control and Prevention CDC (2010). Laboratory detection of oxxacillin/methicillin-resistant Staphylococcus aureus. *National Center for Emerging and Zoonotic Infectious Diese.*

Chambers HF (1997). Methicillin-Resistance in Staphylococci; Molecular an Biochemical Basis and clinical implications. Clin. Microbiol. Rev. 10:781-791

Chambers HF (2001). The changing epidemiology of *Staphylococcus aureus?* Emerg. Infect. Dis. 7:17-82

Chigbu CO, Ezereonye OU (2003). Antibiotic resistant *Staphylococcus aureus* in Abia state of Nigeria. Afr. J. Biotechnol. 2:374-378.

Clinical, and Laboratory Standard Institute, (2007). Performance Standard for antimicrobial susceptibility testing. CLSI approved standard M100-517. *Clinical and Laboratory Standards Institute.* Wayne.

Deurenberg RH, Vink C, Kalenic S (2007). The molecular evolution of Methicillin–resistant *Staphylococas aureus* Clin. microbial Infect. 13:222–235.

Klein E, Smith DL, Laxminarayan R (2007). Hospitalization and Deaths caused by Methicilin–Resistant *Staphylococcus aureus*. United States 1999–2005. Emerg. Infect. Dis. 13:1840–1846.

Kluytmans J, Van Belkum A, Verbrugh H (1997). Nasal carriage of *Staphylococcus aureus:* epidemiology, underlying mechanisms, and associated risks. Clin. Microbial Rev. 10:505–520.

Klevens (2007). Invasive Methicillin–Resistant *Staphylococcus areus* Infections in the United States. *JAMA* 201:360-372.

Noskin GA, Rusin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Smulders M, Lapetina E, Gemmene (2005). The boding of *Staphylococcus aureus* infection on Hospitals in the United States: Analysis of the 2000 and 2001 Nationwide inpatient sample Data base. Ach. Int. Med. 165:1756–1761.

Noskin GA, Rubin RJ, Schentag JJ (2007). National trends I *Staphylococcus are aureus* infection rates: impact on economic binder and mortality over a 6–years period Clin. Infect. Dis. 28:331-342.

Proctor RA (2008). Role of folate antagonists in the treatment of methicillin resistant *Staphylococcus aureus Infection* Clin. Infect. Dis. 46:584–593.

Reuters (2009). "Study: Beachgoers More Likely to Catch MRSA". FoxNews.com.

Rubin RJ, Harrington CA, Poon A, Dietrich K, Greene JA, Mioduddin A (1999). The Economic impact of *Staphylococcus aureus* infection in New York City hospitals. Emerg. Infect. Dis. 5:9–17.

Smith TL, Pearson ML, Wikox KR (1999). For the Glycopeptide – intermediate *Staphylococcus aureus* N. Engl. J. Med. 340:493-501.

Stein R (2007). Drug-resistant staph. germ's toll is higher than thought. *Washington Post.* 41:74-77.

Taiwo SS, Onile BA, Akanbi AA (2004). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Ilorin, Nigeria. Afr. J.Experimental Microbiol. 5:189-199.

Uaboi-Egbenni PO (2003). Incidence of *Staphylococcus aureus* among healthy humans in Lagos and its Environs. Nigeria J. Microbiol. 17:162-172.

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Full Length Research Paper

In vitro discovery of highly chelatable root extract of thorn apple (*Datura stramonium*)

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This study investigated the *in vitro* chelating ability, antioxidant activity, total phenolic and flavonoid contents of 70% ethanolic extract of thorn apple (*Datura stramonium*). The ethanolic root extract of the plant displayed potent chelating ability (70%) at the lowest concentration (2%) investigated. The chelating ability of the extract showed concentration dependency. There were significant decreases *in vitro* chelating ability of the root extract from 2 to 10% concentrations (P<0.05). The root extract also demonstrated exhibited concentration dependent increases in antioxidant activity, which were non-significant from 2 to 6% concentrations (P>0.05) and significant from 8 to 10% (P<0.05). The ethanolic root extract displayed maximum activity [106.20±0.84% standard deviation (SD)] at the highest concentration (10%). The preliminary phytochemical screening of the aqueous extract of *D. stramonium* revealed the presence of flavonoids, tannin and alkaloid, but saponin was absent. The aqueous root extract of the plant also exhibited potent antioxidant potential (71.00%) at 2% concentration. The antioxidant activity of the aqueous root extract of the plant closely approximated the antioxidant activity of the ethanolic root extract of the plant (71.00 and 71.40%, at different concentrations, 2 and 5%, respectively). The aqueous root extract of the plant showed good *in vitro* nitric oxide radical scavenging activity (67.80%). The absolute and 70% acetone root extracts of the plant were dull nitric oxide scavengers (33.38 and 26.00%, respectively). The total flavonoid and phenolic contents of ethanolic root extract of *D. stramonium* showed non-significant increases as the concentration increased (P>0.05). The ethanolic root extract failed chelate ion at the highest concentration (10%) (-20.0% activity).

Key words: Phytotoxicology, clinical toxicology, iron-overload, clinical medicine, natural product.

INTRODUCTION

Polyphenols and flavonoids are used for the prevention and cure of various diseases which is mainly associated with free radicals (Havesteen, 1983). *Datura stramonium* belongs to the family Solanaceae (Oseni et al., 2011). The plant is a drug of abuse (Aroulou et al., 2003). The eating and chewing of the plant is a suicide attempt (Monteriol et al., 2007). It is more commonly called the jimson weed or thorn apple (Abdollahi et al., 2003). It is a wild growing flowering plant (Abdollahi et al., 2003). The plant contains atropine alkaloids such as scopolamine, hyosciamine and atropine primarily in the seed, flower

(Preissel and Hans-George, 2002). The seed oil of the plant is a good source of protein and nutritionally valuable minerals (Oseni et al., 2011).

The seed possessed hallucinogenic and euphoric effects (Ertekin et al., 2005). It contains three main toxic alkaloids atropine, scopolamine and hyosciamine (Urich et al., 1982). The foliage and the seed of the plant are particularly toxic (De Fratels, 2005). The seed extract induced centrolobular necrosis and dilated central vein in rats (Bouzidi et al., 2011).

The seed extract of the plant possessed agglutination

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effect on erythrocytes from several species, and is non-specific with regard to human ABO blood group (Kalpatrict et al., 1978). The aqueous leaf extracts enhanced cytotoxicity on human cancer cells via oxidative stress (Ahmad et al., 2009). The seed extract possessed analgesic effect against acute and chronic pain (Khalili and Atyabi, 2004). Traditionally, the seed extract is used in the treatment of toothache and fever (Sandoval, 1998). Exposure to the smoked extract of the leaf of the plant has deleterious effects on the cytoarchitecture of liver, heart, kidneys and testes in animals (Adekomi et al., 2011). The plant is used as a phytoremediator of explosives and to clear 2,4 ,6- trinitrotoluene via nitro reduction in municipal waste sites (Lucero et al., 1999). Traditionally, smoked leaves of the plant are used as an anti-spasmodic in the treatment of asthma (Foster and Duke, 1990). The root extract mixed with latex of Calotroprocera is used to cure scorpion sting and snake bite (Jain et al., 2011; Najafabadi and Atyabi, 2004).

The methanolic extract, ethanolic and aqueous extracts of the leaves, stem and root showed antibacterial activity (Akharaiyi, 2011; Iranbakhsh et al., 2010). The methanolic extract of the plant showed antioxidant activity (Mishra et al., 2011).

MATERIALS AND METHODS

Collection of plant

The root of *D. stramonium* was collected from the back of Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Oyo State, Nigeria on 4th June, 2011.

Preparation of plant extracts

The plant was washed with distilled water and then cut into pieces. Five different concentrations (2- 10%, w/v) of the root of *D. stramonium* were prepared in 70% ethanol. This was carried out by soaking 2, 4, 6, 8 and 10 g of the root in 100 ml of 70% ethanol in five different containers with lid for 30 min. Each mixture was filtered with Whatman filter paper. Each filtrate was used for biochemical assays of interest.

Another 5% extract (w/v) of the root of the plant was prepared by soaking 5 g of the root of the plant in 100 ml of each of the solvents used (water, 70% ethanol, absolute acetone and 70% acetone). The mixture was filtered using Whatman filter paper. The filtrate obtained was used for assays of interest.

Chemicals and reagents

The phenanthroline used was a product of British Drug House (BDH), UK. Folin-Ciocalteu reagent used was a product of Merck, Germany. The stable free radical utilized (2,2-diphenyl -1-picrylhydrazyl (DPPH)) was a product of Sigma-Aldrich, USA.

In vitro assays

In vitro Fe^{2+} chelating ability assay

The *in vitro* Fe^{2+} chelating ability of the plant extract was assayed according to the modification of Minnoti and Aust (1987). Briefly, 200 µl of sample was mixed with 150 µl (FeSO$_4$, 500 µM) (freshly prepared), 168 µl of Tris HCl (0.1 M, pH 7.4) and 218 µl of saline (NaCl, 0.9% w/v). The mixture was incubated for 5 min, after which 13 µl aqueous phenanthroline (0.25%, w/v aqueous) was added. The absorbance of the solution was read at 510 nm with distilled water as blank on a spectrophotometer. The *in vitro* of Fe^{2+} chelating ability of the sample was calculated by using the following formula:

Chelating ability (%) = (A$_{control}$ - A$_{sample}$)/A$_{control}$ × 100

where A$_{control}$ is the absorbance of the control (reaction mixture in the absence of sample) (FeCl$_3$ alone); A$_{sample}$ is the absorbance of sample.

In vitro nitric oxide radical scavenging potential assay

The *in vitro* nitric oxide scavenging activity was estimated according to the method of Marcocci et al (1994). To 1 ml sample, 1 ml of sodium nitroprusside (10 mM, aqueous) and 1 ml buffer (sodium phosphate buffer, 0.2 M) were added. The mixture was incubated at room temperature for 150 min (2 h 30 min) followed by the addition of 01 ml Griess reagent. The absorbance of the pink colour solution was read at 540 nm on a spectrophotometer. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with N-naphthyl ethylene diamine dihydrochloride (NED) was measured spectrophotometrically at 540 nm.

The *in vitro* nitric oxide scavenging activity of the sample was calculated by using the following formula:

Nitric oxide scavenging activity (%) = (A$_{control}$ - A$_{sample}$)/A$_{control}$ × 100

In vitro antioxidant activity (DPPH based) assay

The *in vitro* antioxidant activity of the sample was quantitated according to the traditional method of Blois (1958). To 1 ml of plant extract, 1 ml of methanolic solution of DPPH (0.2 mM) was added. The mixture was incubated in the dark for 30 min. The absorbance of the yellow colour solution was read at 517 nm on a spectrophotometer using distilled as blank. This spectrophotometric assay uses the stable radical DPPH as a reagent. The purple colour of the methanolic DPPH which resembles the colour of KMnO$_4$ was changed to yellow colour in the presence of hydrogen or electron donating antioxidant, giving diphenyl picryl hydrazine as a product. DPPH reacts with reducing agents and then electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up (Blois, 1958).

DPPH scavenged (%) = (A$_{DPPH}$ - A$_{sample}$)/A$_{DPPH}$ × 100

A$_{DPPH}$ is the absorbance of methanolic solution of 2,2-diphenyl-1-picryl hydrazyl; A$_{sample}$ is the absorbance of sample.

Total phenolic content assay

The phenolic content of the sample was determined according to the method of Taga et al. (1984). To 0.1 ml of sample, 2 ml of sodium carbonate solution (0.2% w/v) was added, followed by the addition of 0.1 ml of Folin-Ciocalteu reagent (10%, v/v). The mixture was incubated for 10 min. The absorbance of the blue colour solution was read at 480 nm. This is based on chemical reduction of tungsten and molybdenum oxides affording a blue colour solution, which was measured spectrophotometrically. The of total phenolic

Table 1. Changes in levels of phenolics, flavonoids, antioxidant and chelating abilities of 70% root ethanolic extract of Siam weed (*Datura stramonium*).

Concentration (%, w/v)	Phenolic content (mg/ml)	Flavonoid content (mg/ml)	Chelating ability (%)	Antioxidant activity (%)
2	12.20 ± 0.10	2.00 ± 0.10	70.00 ± 1.23	71.41 ± 0.89
4	12.80 ± 0.12	2.60 ± 0.07	40.00 ± 1.23	74.00 ± 1.0
6	13.80 ± 0.14	3.20 ± 0.19	20.00 ±1.00	87.80 ± 0.84
8	14.60 ± 0.07	4.80 ± 0.27	25.00 ±0.71	90.40 ± 0.55
10	15.20 ± 0.12	5.40 ± 0.20	-20.00 ± 1.22	106.20 ± 0.84

Table 2. Changes in the levels of antioxidant and nitric oxide scavenging activities and flavonoid content in 5% aqueous, absolute and 70% acetone extracts of *Datura stramonium* extracts.

Solvent	Flavonoid content (mg/ml)	Antioxidant activity (%)	Nitric oxide radical Scavenging (%)
Water	15.20 ± 1.10	71.00 ± 1.00	67.80 ± 0.84
Absolute acetone	5.20 ± 1.10	68.40 ± 1.14	33.38 ± 0.91
70% acetone	4.40 ± 0.89	70.40 ± 0.89	26.00 ± 0.71

concentration (mg/ml) in the extract was extrapolated from pyrocatechol calibration curve.

Total flavonoid content assay

The flavonoid content of the sample was determined according to the method of Lamaison and Carnet (1990). To 0.5 ml sample, 0.5 ml of 70% $AlCl_3.6H_2O$ (2%) was added and the mixture incubated for 10 min. The absorbance of the yellow colour solution was read at 430 nm after 10 min on a spectrophotometer using distilled water as blank. The total flavonoid concentration (mg/ml) of the extract was obtained from a calibration curve using quercetin as a standard flavonoid. In this colorimetric assay, the $AlCl_3$ acid stable complexes with the 4-keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols also form acid labile complexes with the ortho-dihyroxyl groups in the A or B-ring of flavonoids.

RESULTS

The ethanolic root extract of the plant showed potent chelating ability (70.0% activity) at the lowest concentration (2%). The ethanolic extract of the plant displayed excellent antioxidant activity (106.20% at the highest concentration, 10%). The *in vitro* chelating potential ranged from -20.0 to 70.0% (Table 1). The total flavonoid and phenolic contents of the ethanolic root extracts showed non-significant increases as the concentration of the plant extract increased (P>0.05). The maximum total phenolic concentration of the ethanolic root extract was 15.20 mg/ml. At the highest concentration, the ethanolic root extract of *D. stramonium* failed to chelate ion *in vitro*. The antioxidant activity of the aqueous root extract of the plant closely approximated the antioxidant activity of the ethanolic root extract of the plant (71.00 and 71.40%, at different concentrations, 2 and 5%, respectively) (Tables

1 and 2).

The order of *in vitro* nitric oxide, radical scavenging activity of *D. stramonium* selected was investigated: water > absolute acetone > 70 acetone. The *in vitro* nitric oxide radical scavenging activity of the aqueous of the plant was significantly higher than that of 70% acetone extract (P<0.001). The 70% and absolute root extracts of *D. stramonium* were weak *in vitro* nitric oxide scavengers (26.0 and 33.38% nitric oxide scavenging potential, respectively). The order of flavonoid content in selected solvents (water, absolute and 70% acetone) was: water > absolute acetone > 70% acetone acetone. The *in vitro* nitric oxide scavenging activity of absolute ethanolic extract of the plant was higher than the 70% acetone extract, but the difference was not significant (P>0.05). In addition, the aqueous, absolute and 70% acetone extracts of the plant demonstrated potent antioxidant acti-vity. The antioxidant activity of the aqueous extract of the plant and the 70% acetone extract was in the region of 70%. The values of *in vitro* antioxidant potentials of the absolute and 70% acetone extracts were significantly higher than *in vitro* nitric oxide radical scavenging in the two solvents (P<0.001).

Phytochemical screening revealed that saponin was absent in the aqueous root extract of the plant. Flavonoid, tannin and alkaloids were detected in the aqueous extract of the plant (Table 3).

DISCUSSION

Chelation therapy is the preferred medical treatment for reducing the toxic effects of metals (Flora and Pachauri, 2010). Metals with normal concentration have essential roles in body metabolism; however, in higher concentration

Table 3. Preliminary screening of bioactive constituents of aqueous root extract of *D. stramonium*.

Phytochemical	Solvent (Distilled water)
Flavonoid (Shibata's test)	+
Tannin	+ +
Alkaloid (Meyer's test)	+ +
Saponin (Frothing test)	-

[+]Indicates slightly positive, while [++]Indicates strongly positive.

concentration they can induce severe toxicity (Mirzaei and Khatami, 2013). A sample high in polyphenol might not chelate metal ions if the polyphenol present did not possess suitable groups that could chelate the metal cations (Hider et al., 2001). Bidentate ligands are more powerful scavengers of metal cations than monodentate ligands (Hider et al., 2001). Chelating agents are effective as secondary antioxidants (Gordon 1990).

The lowest concentration of the 70% root ethanolic extract of Siam weed (*D. stramonium*) (2%) displayed potent chelating ability *in vitro*. This is the first report of *the in vitro* discovery highly chelatable extract of the plant in 70% ethanol. The value obtained in the present study was low when compared with the maximum chelating ability of hydroalcohol extract of *Coriander sativum* (90%) previously reported (Mirzae and Khatami, 2013). Our experimental value for chelating ability of *D. stramonium* extract *in vitro* state (70%) was the same to that of chelating ability of aqueous stem extract of *Euphorbia macrolada* (70%, at 1 mg/ml) reported by Farhan et al. (2013). In the present study, the ethanolic root extract of *D. stramonium* showed concentration dependent decrease in chelating ability as the concentration of the extract increased, which was consistent with research work on chelating ability of *Bauchinia purpurea* stem bark extracts with ethyl acetate, petroleum ether and methanol as extraction solvents (Chaudhari and Nagar, 2013).

It is known that the ability of phenolic compounds to chelate metal ions depends on the availability of properly oriented functional groups (Van-Acker et al., 1996). Phytochemical screening of the aqueous extract of *D. stramonium* in our present work revealed the presence of flavonoids and tannins, which was consistent with some phytoconstituents of the ethanolic leaf extract of *Phyllanthus amarusschonn* earlier reported (Ujwala et al., 2012). The phytochemicals evaluations of plants which have suitable use in folkore have often resulted in the isolation of principles with remarkable bioactivities (Afolabi et al., 2007). Flavonoids are known to exhibit antioxidant properties through chelating with transition metals, primarily Fe^{2+}, Fe^{3+} and Cu^{2+}, which participate in reactions generating free radicals (Malesev and Kuntic, 2007). Flavonoids constitute a large group of polyphenolic compounds with antioxidant properties which are overwhelmingly exerted through direct free radical scavenging (Malesev and Kuntic, 2007). Antioxidant activity of phenolic compounds is due to their tendency to chelate metals (Michalak et al., 2006).

Plant extracts offer promising sources of natural antioxidants (Tupe et al., 2003). The *in vitro* antioxidant activity of the ethanolic carrot (*Daucus carota*) root crude extract (27.5%) (Chatatikun and Chiabchalard, 2013) was lower than the maximum antioxidant activity of the root ethanolic extract (106.20%) observed in the present study at the highest concentration of the plant (10%). The phenolic compounds may contribute directly to antioxidative action (Awika et al., 2003). Rutin is a standard flavonoid and exhibited *in vitro* antioxidant activity (69.83%) at 1000 µg/ml (Kumar et al., 2010). However, the antioxidant activity of root ethanolic extract *D. stramonium* was obtained in this work (106.20%) at 10% concentration greater than that of rutin. It has been reported that radical scavenging action is dependent on both the reactivity and concentration of the antioxidant (Resat et al., 2007). The antioxidant activity of an antioxidant compound has been attributed to various mechanisms among which are the binding of transition metal ion catalysts, prevention of hydrogen abstraction and radical scavenging (Gulcin et al., 2005). DPPH is usually used as a substrate to evaluate the antioxidant activity of antioxidants (Duh et al., 1999).

The reduction of nitric oxide with phenolic groups present in antioxidant may serve to attenuate the concentration of nitric oxide (Wilcox and Janzen, 1993). Nitric oxide radical scavenging capability of ascorbic acid *in vitro* state (95%, at 3 or 5 mg/ml) reported elsewhere (Chai et al., 2013) was greater than our experimental values (67.80, 33.38 and 26% for water, absolute acetone and 70% acetone, respectively).

REFERENCES

Adekomi DA, Musa AA, Tijani AA, Adeniyi TD, Usman B (2011). Exposure to smoke extract of *Datura stramonioum* leaf; some of its effects on the heart, liver, lungs, kidney and testes of male Spraque Dawley rats. J. Pharmacogn. Phytother. 3(5):67-75.

Ahmad IM, Maher YA, Noor HM, Esam YQ, Fuad AA (2009). Datura aqueous leaf extract enhances cytotoxicity via metabolic oxidative stress on different human cancer cell lines. J. Biol. Sci. 2(1):9- 14.

Akhaiji FC (2011). Antibacterial, phytochemical and antioxidant activities of Datura metel. Int. J. Pharm. Tech. Res. 3(1):478-483.

Aroukou H, Matray MD, Braganca C, Mpaka JP, Chinello L, Castaing F, Barton C, Poisot D (2003). Voluntary poisoning by ingestion of *Datura stramonium*. Another cause of hospitalization in youths seeking strong sensations. Ann. Med. Int. 154:46- 50.

Awika JM, Rooney LW, Wu X , Prior RL , Cisneros-Zevallos L (2003). Screening methods to measure antioxidant activity of (*Sorghum bicolor*) and sorghum products. J. Agric. Food Chem. 51:6657–6662.

Blois MS (1958). Antioxidant determination by using a stable free radical. Nature 181:1199–1200.

Bouzidi A, Mahdeb N, Kara N (2011). Toxicity studies of alkaloids of seeds of *Datura stramonium* and synthesis alkaloids in male rats. J. Med. Plants Res. 5(15):342 -3431.

Chai T, Uah Y, Ooh K, Ismail NIM, Ang Y, Elamparuthi S, Yeoh L, Ong H, Wong F (2013). Anti-proliferative, antioxidant and iron –chelating properties of the tropical highland fern, *Phymatopteris triloba* (Houtt) Pichi serm family (Family Polypodiaceae). Trop. J. Pharmaceut. Res. 12(5):747-753.

Chatatikun M, Chiabchalard A (2013).Phytochemical screening and free

radical scavenging activities of orange baby carrot(Daucus carota Linn.) root crude extracts . J. Chem. Pharmaceutical Res. 5(4):9–102.

Chaudhari M, Nagar NVV (2013). *In vitro* screening of Bauhinia purpurea stem bark with reference antioxidant activity. Int. J. Res. Humanit. Soc. Sci. 1(6):37–50.

Duh PD, Tu YY, Yen GC (1999). Antioxidant activity of aqueous extract of Hamjyur (*Chrsanthemum morifolium* Ramat). Lebensmwiss Technol. 32:269–277.

Ertekin V, Selimogly MA, Altinkaynak SA (2005). A combination of unusual presentations of *Datura stramonium* intoxication in a child with rhabdomylysis and fulminant hepatitis. J. Energy Med. 28:227-228.

Farhan H, Rammal H, Hijazi A, Daher A, Reda M, Annan H, Chokr A, Bassal A, Badran B (2013). Chemical composition and antioxidant capacity of a Lebanese plant *Euphorbia macrolada* Schyzoceras. Asian Pac. J. Trop. Biomed. 3(7):542–548.

Flora SJS, Pachauri V (2010). Chelation in metal intoxication. Int. J. Environ. Res. Public Health 7(7):2745-2788.

Foster S, Duke JA (1990). Field guide to medicinal plants in Eastern and central America. Peterson RT (ed). Houghton Mifflin Company, Boston, USA. P 432.

Gordon MH (1990).The mechanism of antioxidant action *in vitro*. In: Hudson BJF (ed.), Food Antioxidants. Elsevier Applied Science, London, UK. pp 1–18.

Gulcin I, Alici HA, Censur M (2005). Determination of *in vitro* antioxidant and radical scavenging activities of propofol. Chem. Pharm. Bull. 53:281-285.

Havesteen B (1983). Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol. 30:1141–1148.

Hider RC, Liu ZD, Khodn HH (2001). Metal chelation of polyphenols. Methods Enzymol. 335:190 – 203.

Iranbakhsh A, Ebali M, Bayat M (2010). The inhibitory effects of plant methanolic extract of *Datura stranmonium* L. and leaf explants callus against bacteria and fungi. Glob. Veterinaria 4(2):149–155.

Jain Y, Verma SK, Katewa SS, Anandjiwala S, Singh B (2011). Free radical scavenging property of bomb ax Ceiba Linn. Root Res. J. Med. Plant 5(4):462-470.

Khalili NM, Atyabi SM (2004). Evaluation of analgesic effect of *Datura stramonium* seed extract in hot plate and formalin tested on male rats. Int. J. Med. Arom. Plants Res. 20(3):309–322.

Kumar DS, Muthu AK, Smith AA, Manavalan R (2010). In vitro antioxidant activity of various extracts of whole plant of *Mucuna pruriens* (Linn.) .Intl. J. Pharm. Tech. Res. 2(3):2063–2070.

Lamaison JLC, Carnet A (1990). Teneurs en pricipaux flavonoids des fleurs de Crataegeus monpgyna Jacg et de Crataegeus laevigata(Poire DC) en fonction de la vegetation.Pharma Acta Helv 65:315-320.

Lucero ME, Mueller W, Hubstenberder J, Philips GC, O'Conell MA (1999).Tolerace to nitrogenous explosives and metabolism of TNT by cell suspensions of Datura innoxia.In vitro Cell Dev Plant 35:480-486.

Malesev D, Kuntic V (2007). Investigation of metal -flavonoid chelates and the determination of flavonoids via metal –flavonoid complexing reactions. J. Serb. Chem. 72(10):921-939.

Marcocci L, Maguire JJ, Drog-Lefix MT, Packer L (1994).The nitric oxide scavenging properties of *Gingko biloba* extract EGb. Biochem. Biophys. Res. Commun. 201:748–755.

Minnoti G, Aust SD (1987). An investigation into the mechanism of citrate –Fe^{2+} dependent lipid peroxidation. Free Rad. Biol. Med. 3:379-387.

Mirzae A, Khatami R (2013). Antioxidant and iron chelating activity of *Coriander sativum* and *Petroselinum crispum*. Bull. Environ. Pharmacol. Life Sci. 2(7):27-31.

Mishra SS, Patel KK, Raghuwanshi N, Pathak A, Panda PP, Girhepunju K, Patro CN (2011). Screening of ten Indian medicinal plant extracs for antioxidant activity. Ann. Biol. Res. 2(1):162-170.

Monteriol A, Kenane N, Delort G, Asencio Y, Palmier B (2007). Intentional *Datura stramonium* intoxication: an unknown etiology of mydriasis. Ann. Fr. Anesthesiol. Reanim. 26:810-813.

Oseni OA, Olarinoye CO, Amoo IA (2011). Studies on chemical composition and functional properties of thorn apple (*Datura stramonium* L.) Solanaceae. Afr. J. Food Sci. 5(2):40–44.

Preiseel U, Hans-George P (2002). Brugmansia and Datura: Angel's Trumpets and Thorn Apples Buffalo, New York .Fire –Fly Books; pp 106–129.

Resat A, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B, Berker KI, Ozyurt D (2007). Comparative evalutation of various total capacity assays applied to phenolic compounds with CUPRAC assay.Molecules 12:1496-1547.

Taga MS, Miller EE, Pratt DE (1984). Chia seeds as a source of lipid antioxidants. J. Am. Oil. Chem. Soc. 61:928-931.

Tupe KS, Kemse NG, Khaire AA (2013). Evaluation of antioxidant potentials and total phenolic contents of selected Indian herb powder extracts. Intl. Food Res. J. 20(3):1053–1063.

Ujwala W, Vijender S, Mohammed A (2012). *In vitro* antioxidant activity of isolated tannins of alcoholic extract of dried leaves of *Phyllanthus amarusschonn* and Thonn. Intl. J. Drug Dev. Res. 4(1):274–285.

Urich RW, Bowerman DL, Levisky JA, Pflug JL (1982). *Datura stramonium*: a fatal poisoning. J. Forensic Sci. 27(4):948–954.

Van-Acker SA, Van-den Berg BE, Tromp DJ, Van-der Vijgh WJ, Griffioen DH (1996). Structural aspect of antioxidant activity of flavonoids. Free Rad. Biol. Med. 20:331-342.

Wilcox AL, Janzen EG (1993). Nitric oxide reactions with antioxidants on model systems: sterically hindered phenols and α-tocopherol in sodium dodecyl sulphate (SDS) micelles. J. Chem. Soc. Chem. Commun. 18:1377–1379.

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An assesment of the spatial pattern of malaria infection in Nigeria

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Malaria transmitted by female anopheles mosquitoes is a major cause of death in many developing countries of the world. In Nigeria, malaria prevalence is as high as 80 to 85% and is the most common cause of outpatient visits to health facilities. The malaria situation in Nigeria is very burdensome and it impedes human development. The degree of malaria infestation varies from region to region in Nigeria. This spatial attribute of malaria infestation across regions necessitate the needs for malaria mapping among researchers. Also, the rate of malaria infection across space depends on dynamic processes involving complex climatic, environmental, physical, and social variables operating differently in space. This complexity makes the analysis of the spatial pattern of malaria infection in Nigeria important. Such analysis can explain the variations, providing a basis for policy intervention. It is against this background that this paper examines the spatial patterns of malaria infestation in Nigeria. Malaria data for fifteen years (1993 to 2007) were collected from the World Health Organisation (WHO) Data Bank, Roll Back Malaria/Epidemiological Unit of both the Federal and State Ministries of Health for twenty-three states in Nigeria. The pattern of spatial variation in the rate of malaria infection was analyzed using principal component analysis (PCA). The results indicate that seasonal variations play significant roles in malaria infection in Nigeria. It also shows high concentration of malaria infections in some few states. This paper therefore recommends that deliberate effort should be made to increase the distribution of treated mosquito nets and drugs in the affected states and an increment in the financial allocation to the affected states by the Federal Ministry of Health with a few to reducing the effect of the disease in the affected states.

Key words: Assessment, spatial, patterns, malaria, infection, Nigeria.

INTRODUCTION

Malaria is a parasitic disease transmitted by female anopheles mosquitoes. Malaria affects 3.3 billion people, or half of the world's population, in 106 countries and territories. World Health Organisation (WHO) estimated 216 million cases of malaria in 2010, 81% in the African region. WHO estimated that there were 655,000 malaria deaths in 2010, 91% in the African region, and 86% were children under 5 years of age. Malaria is the third leading cause of death for children under five years worldwide, after pneumonia and diarrheal disease. Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigeria's population. The remaining 3% of the population live in the malaria free highlands. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215,000 deaths per year in Nigeria from HIV/AIDS. Malaria contributes to an estimated 11% of maternal mortality (Akpan, 1996; Thompson, 2004; US Embassy Nigeria, 2011; United States

Agency for International Development [USAID], 2011; National Population Commission (NPC) [Nigeria], National Malaria Control Programme (NMCP) [Nigeria], and ICF International, 2012).

The malaria situation in Nigeria is very burdensome and it impedes human development. It is both a cause and consequence of underdevelopment (Department for International Development [DFID], 2008). The degree of malaria infestation varies from region to region in Nigeria. This spatial attribute of malaria infestation across regions necessitate the needs for malaria mapping among researchers. The mapping of patterns in the spatial distribution of features has been of great significance in virtually all fields. The primary aim in the mapping process is to bring out hidden relationships among variables (Oluwafemi et al., 2013). Detailed mapping of malaria in Africa using actual malaria data have been very difficult due to paucity of data, thus the use of climatic models, which can predict fairly accurately, the real situation, is normally used. In addition, most of the researches on malaria mapping in sub-Saharan Africa have been concentrated in East and Southern Africa, Craig et al. (1999) in Kenya, Hay et al., (2002) in East African Highlands, Lindsay and Birley (1996) in Tanzania, Lindsay and Martens (1998) in Zimbabwe, Votava et al. (2000) in Burundi and Malawi. Little or nothing have been done in West Africa especially Nigeria. Though, malaria mapping/modelling using climatic variables tends to explain the malaria situation in a fairly accurate way, these models are mostly theoretical and they are normally based on available long-term climatic data (Martens et al., 1995; Shanks et al., 2002). But weather conditions and therefore malaria transmissions, vary substantially from one year to the next and from region to region. This makes the inclusion of actual malaria data from the field very fundamental in malaria mapping studies, as they are useful for understanding trends in the relative burden of malaria in the public health sector (WHO Malaria Country Report, 2005; Idowu et al., 2009).

In addition, the rationale behind malaria mapping is centred on the fact that malaria vector is distributed unevenly both within and between places, that is, it exhibits spatial variation. The rate of malaria infection across space depends on dynamic processes involving complex climatic, environmental, physical, and social variables operating differently in space. This complexity makes the analysis of the spatial pattern of malaria infection in Nigeria important. Such analysis can explain the variations, providing a basis for policy intervention. It is against this background that this paper examines the spatial patterns of malaria infestation in Nigeria.

MATERIALS AND METHODS

Malaria data for fifteen years (1993 to 2007) were collected from the WHO Data Bank, Roll Back Malaria/Epidemiological Unit of both the Federal and State Ministries of Health for twenty-three states in Nigeria. The year 1993 to 2007 was used due to the availability of

data in the period. Total malaria infections for each month for the fifteen year period were added up and the aggregated values for January to December for the fifteen year period represent the variables of the study. Consequently, there are twelve variables (January to December). The pattern of spatial variation in the rate of malaria infection was analyzed using principal component analysis (PCA). PCA is a branch of factor analysis used to reduce many related variables into a few underlying constructs without losing their statistical validity. PCA has 3 principal objectives namely: (a) to reduce large set of data to a manageable size; (b) to identify presumed factors that underlie a large set of factors; and (c) to test hypothesis about the relationship among variables (Dillion and Goldstein, 1984).

The first step in PCA is the standardization of all the variables to produce a matrix structure. In this study, the data were standardized using the Z-score transformation technique. The second step involves correlation analysis. In this context, a multiple correlation analysis was computed from the standardized data matrix. The correlation matrix indicates the degree of inter-correlation among the variables, with elements along the diagonals indicating the total variations in the population represented. The output of the correlation shows that some of the variables were highly correlated with each other. In the light of this correlation observed, the principal component was computed using varimax rotation.

FINDINGS

Of the total twelve variables that were used for the analysis, three critical components that had greater than one eigenvalue were identified. These three components represent three structural patterns in malaria infestation during the period under investigation. It explains the spatial variation in the pattern of malaria infestation across the 23 states used for the study. Table 1 shows the eigenvalue table. The eigenvalue table shows the number of components retained from the varimax rotation.

Table 1 shows that the three components combined accounts for approximately 77% of the total variance (this value can be observed at last column with the heading label cumulative percentage). The major criteria used in retaining the ten components are the eigenvalue greater than one, the screen plot technique and the percentage variance accounted for criterion. The first three components retained have their eigenvalue greater than one. The first component accounted for the highest percentage (39.76%) of the total variance explained by the total variables. Component two accounted for 27.28%, while component three accounted for 9.97%. Also, the first component accounted for the highest eigenvalue. It accounted for 4.77 eigenvalue. Component two accounted for 3.27 eigenvalue, while component three accounted for 1.19 eigenvalue. The fourth component down to the twelfth components has an eigenvalue less than one and hence they were not used for interpretation of the result.

So far, the results from the eigenvalue-one criterion, the variance accounted for criterion, and the screen plot converged in suggesting that a three-component solution is appropriate. It is now time to review the rotated component

Table 1. Eigenvalue.

Component	Initial eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)
1	4.772	39.763	39.763	4.772	39.763	39.763	4.654	38.785	38.785
2	3.273	27.277	67.039	3.273	27.277	67.039	3.254	27.117	65.902
3	1.196	9.968	77.008	1.196	9.968	77.008	1.333	11.105	77.008
4	0.668	5.566	82.573	-	-	-	-	-	-
5	0.629	5.241	87.815	-	-	-	-	-	-
6	0.496	4.135	91.949	-	-	-	-	-	-
7	0.322	2.687	94.636	-	-	-	-	-	-
8	0.251	2.090	96.726	-	-	-	-	-	-
9	0.189	1.575	98.302	-	-	-	-	-	-
10	0.123	1.025	99.326	-	-	-	-	-	-
11	4.245E-02	0.354	99.680	-	-	-	-	-	-
12	3.840E-02	0.320	100.000	-	-	-	-	-	-

Extraction method: Principal component analysis.
Source: Author's Compilation (2012).

Table 2. Rotated component matrix of malaria infection in some selected states in Nigeria.

Month	Component 1	Component 2	Component 3
January	0.893*	0.167	0.111
February	0.877*	5.196E-02	0.157
November	0.869*	-0.152	1.977E-02
March	0.843*	0.197	6.498E-02
October	0.791*	-0.171	0.117
December	0.763*	-0.157	2.714E-02
May	-6.849E-02	0.921*	-0.113
June	-0.291	0.880*	-0.111
July	-9.124E-03	0.825*	0.352
April	0.431	0.671*	-0.132
August	0.146	0.594*	0.487
September	0.152	-3.539E-02	0.935*

Extraction method: Principal component analysis. Rotation method: Varimax with Kaiser normalization. a Rotation converged in 4 iterations.
Source: Author's Compilation (2012).

component matrix pattern to see if such a solution is interpretable. This matrix is presented in Table 2. The entries in the table are the factor loadings. They represent clusters of inter-related variables, which delineate general patterns of covariations within the data set. Each of the variables is weighted according to its degree of importance in defining the principal components. The second line of column two is the factor loading of 0.893 for the variable "January" on component one. In the third column of the same row is a factor loading of 0.167 for the variable "February" for component two. Thus, each of the variables loaded on the three retained components.

In Table 2, six variables have their highest loadings on component one. They are January, February, March, October, November and December. Five variables have their highest loadings on component 2. They are April, May, June, July and August. The last component has one variable loaded on it and that is September. The three components retained represent three different patterns or clusters of malaria infestation in Nigeria. The variables in the first component contribute more to the spatial variations in malaria infestation in Nigeria and component two contributes more than component three. From the pattern of the loadings on the three components, it reveals that variables in the first component are all within the dry season period, while the component two variables are the rainy season period. However, the third component represents a transition period between the rainy

Table 3. Components, variables and their labels.

Component	Variable	Label
1	January, February, March, October, November and December	Dry season component
2	April, May, June, July and August.	Rainy Season component
3	September.	Transition season component

Source: Author's Compilation (2012).

Table 4. Component scores of malaria infection in some selected states in Nigeria.

State	Component 1	Component 2	Component 3
Lagos	1.222	0.096	-0.333
Edo	0.936	-0.649	-0.561
Ondo	1.059	-0.898	-0.293
Rivers	0.965	-0.871	-0.331
Enugu	-0.226	-1.414	0.050
Delta	1.099	0.676	-0.347
Imo	0.176	-1.146	-0.120
Cross rivers	0.470	-0.356	-0.706
Oyo	0.980	0.694	-0.274
Katsina	-0.612	0.849	-0.509
Kano	-0.931	1.942	-0.203
Borno	-1.162	1.115	0.362
Sokoto	-0.885	0.481	0.194
Bauchi	-1.045	-0.900	-0.240
Kaduna	-0.721	-0.046	-0.4731
Adamawa	-1.256	-1.717	-0.415
Plateau	-1.164	-1.875	-0.282
Benue	1.616	1.424	0.027
Kwara	-0.365	1.245	-0.814
Kogi	0.262	0.948	0.811
Zamfara	-0.714	0.398	0.258
Yobe	-1.277	0.110	3.979
Niger	1.12	0.340	0.507

Source: Author's Compilation (2012).

Labelling the components

For the purpose of identification, there is the need to identify the various components using appropriate labels. This is important because the derived components have no names. Consequently, each of the three components was labelled as shown in Table 3. The table also indicates the variables that make up each of the components.

Table 3 shows the labels of the three components. Component one with six loadings is label dry season component; component two with five loadings is label rainy season component, while the third component with a single loading is labelled the transition season component. The three components constitute the underlying factors that explain the spatial pattern of malaria infestation in Nigeria. While the aforementioned analysis has determined the relative contribution of the various variables across the three components, their spatial distributions across the twenty-three states which the study covers is yet to be determined. This is achieved by the determination of the component scores of each state for the three retained components. The determination of the component scores permits inter-state comparison of the level of contribution of each of the three components to the twenty-three states. The component scores were obtained by summing the products of the standardized scores and the loading under each component score co-efficient matrix (Table 4).

The scores ranged from 1.61652 for Benue state to –1.27737 for Yobe state. States that display high component scores for component one labelled dry season component include state in the southern part of Nigeria and the middle belt, while states that display low component scores include states in the northern part of Nigeria. For a clearer observation and analysis of the spatial pattern of the dry season component scores, the states were grouped into three categories namely, high level malaria infestation, medium level malaria infestation and low level malaria infestation.

Table 5 shows that three states are located in the high-level malaria infestation in the first group. They are Lagos, Ondo, Delta, Benue and Niger state. The second group labelled medium level infestation is made up of five states including Edo, Rivers, Imo, Cross Rivers and Oyo state, while the last group labelled low level infestation has twelve states including Enugu, Katsina, Kano, Borno, Sokoto, Bauchi, Kaduna, Adamawa, Plateau, Kwara, Zamfara, and Yobe state. The different levels of infestation as identified earlier are reflection of the spatial variations in malaria infestation in Nigeria. The pattern reveals from Table 5 shows that the southern states and middle belt states with higher humidity during the dry season records higher malaria infestation in the first component while the northern states with lower humidity during the dry season records lower infestation in the first component.

The third column in Table 4 shows the second component scores labelled rainy season component for each of the twenty-three states. The scores range from1.94245 for Kano state to –1.8753 for Plateau state. States that display high component scores for component two labelled rainy season component include state in the northern part of Nigeria and the middle belt, while states

Table 5. Level of infestation: dry season component .

Group	Range of score	State	Level of malaria infestation
1	>1.00	Lagos, Ondo, Delta, Benue, Niger	High level infestation
2	1.00-0.01	Edo, Rivers, Imo, Cross Rivers, Oyo and Kogi	Medium level infestation
3	<0.01	Enugu, Katsina, Kano, Borno, Sokoto, Bauchi, Kaduna, Adamawa, Plateau, Kwara, Zamfara, Yobe	Low level infestation

Source: Author's Compilation (2012).

Table 6. Level of infestation: rainy season component (Author's Compilation, 2012).

Group	Range of score	State	Level of malaria infestation
1	>1.00	Kano, Borno, Benue, Kwara	High level infestation
2	1.00-0.01	Niger, Yobe, Zamfara, Kogi, Sokoto, Katsina, Oyo, Delta	Medium level infestation
3	<0.01	Lagos, Edo, Ondo, Rivers, Enugu, Imo, Cross Rivers, Bauchi, Kaduna, Adamawa, plateau	Low level infestation

Source: Author's Compilation (2012).

Table 7. Level of infestation: rainy season component (Author's Compilation, 2012).

Group	Range of score	State	Level of malaria infestation
1	>1.00	Yobe	High level infestation
2	1.00-0.01	Niger, Zamfara, Kogi, Sokoto, Borno	Medium level infestation
3	<0.01	Lagos, Edo, Ondo, Rivers, Enugu, Imo, Cross Rivers, Oyo, Katsina, Kano, Bauchi, Kaduna, Delta, Adamawa, Plateau, Kwara, Benue	Low level infestation

Source: Author's Compilation (2012).

that display low component scores include states in the southern part of Nigeria. Again, for a clearer observation and analysis of the spatial pattern of the rainy season component scores, the states were grouped into three as shown in Table 6.

Table 6 shows that four states are located in the high-level malaria infestation in the first group in the second component labelled rainy season component. They are Kano, Borno, Benue, and Kwara state. The second group labelled medium level infestation is made up of eight states including Niger, Yobe, Zamfara, Kogi, Sokoto, Katsina, Oyo, and Delta state, while the last group labelled low level infestation has eleven states including Lagos, Edo, Ondo, Rivers, Enugu, Imo, Cross Rivers, Bauchi, Kaduna, Adamawa, and Plateau state. The second component shows a mixed pattern among the states contributing to the spatial variation in malaria infes-tation. There is no clear distinction among the southern and northern states in this component. The seasonal variations have less influence in the rate of ma-laria infestation in component two. This partially explains the mixed pattern among the states as observed earlier. This also explains while component two explains only 27.28% of the total variance in malaria infestation as against 39.76 of the first component. The fourth column in Table 4 shows the third component scores labelled transition season component for each of the twenty-three states. The component scores range from 3.97962 for Yobe

state to –0.8147 for Kwara state. Also, for a clearer observation and analysis of the spatial pattern of the rainy season component scores, the states are grouped into three as shown in Table 7.

Table 7 shows that one state is located in the high-level malaria infestation in the first group in the third component labelled transition season component and that is Yobe state. The second group labelled medium level infestation is made up of five states including Niger, Zamfara, Kogi, Sokoto, and Borno, state, while the last group labelled low level infestation has seventeen states including Lagos, Edo, Ondo, Rivers, Enugu, Imo, Cross Rivers, Oyo, Katsina, Kano, Bauchi, Kaduna, Adamawa, Plateau, Kwara, Benue, and Delta state. The third component also shows a mixed pattern among the states contributing to the spatial variation in malaria infestation. There is no clear distinction among the southern and northern states. The climatic factors of rainfall and humi-dity have lesser influence in the rate of malaria infestation among the twenty-three states in component three. This also explains why component three explains only 9.97% of the total variance in malaria infestation among the twenty-three states. To show the total variations in mala-ria infestation, the component scores for each of the state for each of the state from component one to three (Table 4) was added up. This was transformed to z-scores (Table 8). The results show that the z-scores range from1.79 for Benue to -1.99 for Adamawa state. Figure 1

Table 8. Standardized component scores of malaria infection in some selected states in Nigeria.

State	Component 1	Component 2	Component 3	Total	Z-score
Lagos	1.222	0.096	-0.333	0.985	0.57
Edo	0.936	-0.649	-0.561	-0.274	-0.16
Ondo	1.059	-0.898	-0.293	-0.132	-0.08
Rivers	0.965	-0.871	-0.331	-0.237	-0.15
Enugu	-0.226	-1.414	0.050	-0.1.59	-0.94
Delta	1.099	0.676	-0.347	1.428	0.83
Imo	0.176	-1.146	-0.120	-1.09	-0.65
Cross rivers	0.470	-0.356	-0.706	-0.592	-0.35
Oyo	0.980	0.694	-0.274	1.40	0.82
Katsina	-0.612	0.849	-0.509	-0.272	-0.17
Kano	-0.931	1.942	-0.203	0.808	0.47
Borno	-1.162	1.115	0.362	0.315	0.18
Sokoto	-0.885	0.481	0.194	-0.21	-0.13
Bauchi	-1.045	-0.900	-0.240	-2.185	-1.29
Kaduna	-0.721	-0.046	-0.4731	-1.240	-0.73
Adamawa	-1.256	-1.717	-0.415	-3.388	-1.99
Plateau	-1.164	-1.875	-0.282	-3.321	-1.95
Benue	1.616	1.424	0.027	3.067	1.79
Kwara	-0.365	1.245	-0.814	0.066	0.03
Kogi	0.262	0.948	0.811	2.021	1.18
Zamfara	-0.714	0.398	0.258	-0.058	-0.04
Yobe	-1.277	0.110	3.979	2.812	1.65
Niger	1.12	0.340	0.507	1.967	1.15

Source: Author's Compilation (2012).
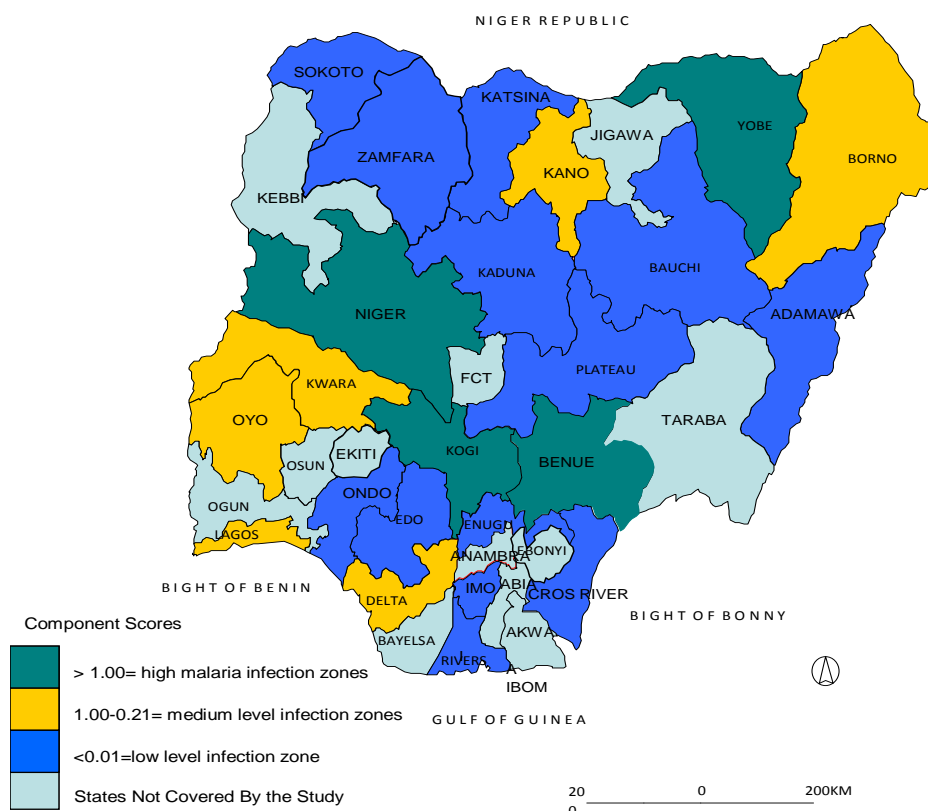
Figure 1. Spatial variations in malaria infestation in Nigeria.

shows the spatial variations in the distribution of malaria infection in Nigeria.

For a clearer observation and analysis of the spatial pattern of malaria infestation in Nigeria, the states were grouped into three categories, namely, high level malaria infestation (>1.00), medium level malaria infestation (1.00 to 0.021) and low level malaria infestation (<0.01). Figure 1 shows the spatial distribution of malaria infections in Nigeria. From Figure 1, four states occupy the high malaria infection zones. They are Kogi, Niger, Yobe and Benue states. Also, six states occupy the medium level malaria infection zones. They are Oyo, Lagos, Kwara, Delta, Kano and Bornu states. In addition, thirteen states occupy the low level malaria infection zones. They are Sokoto, Zamfara, Katsina, Kaduna, Bauchi, Plateau, Adamawa, Ondo, Edo, Enugu, Imo, Cross River and River states.

Conclusion

Seasonal variations play significant roles in malaria infection in Nigeria. Surprisingly, as indicated by the analysis, there are high levels of malaria infestation during the dry season than the rainy season. The paper also indicates that Kogi, Niger, Benue and Yobe states in Nigeria occupies the zones of high malaria infection. States like Oyo, Lagos, Kwara, Delta, Kano and Bornu occupy the medium level malaria infection zones, while states like Sokoto, Zamfara, Katsina, Kaduna, Bauchi, Plateau, Adamawa, Ondo, Edo, Enugu, Imo, Cross River and River occupy the low level malaria infection zones. The concentration of malaria in a few states has specific implications for the health of the people. One consequence of the concentration is loss of income and man hour on the part of infected people, while huge governmental resources are wasted in procuring the required drugs. Deliberate efforts should be made to increase the distribution of mosquito treated nets and drugs in the affected states. Measures should be introduced to increase the financial allocation to the affected states by the federal ministry of health with a few to reducing the effect of malaria infection in the states located in the high infection zones.
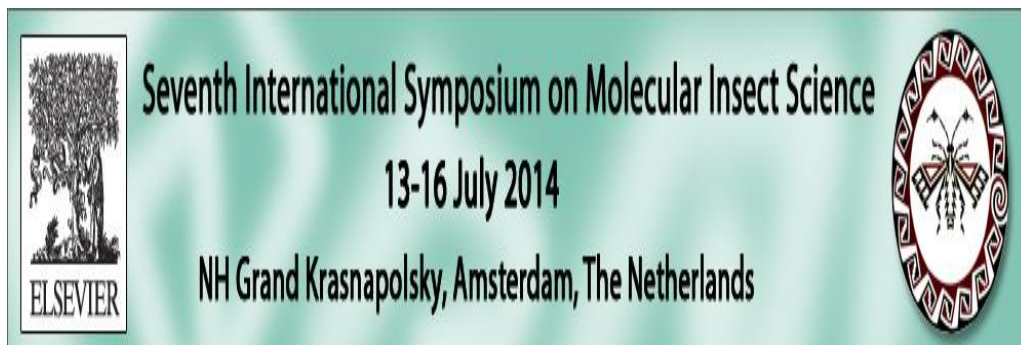
REFERENCES

Akpan HEM (1996). Home Management of Malaria in Children." Mal. Soc. Niger. Newslett. 1(1):8-9.

Craig MH, .Snow RW, Le Seur D (1999). "A Climate-Based Distribution Model of Malaria Transmission in Africa. Parasitol. Today (in Press).

DFID (2008). Rolling Back Malaria in Nigeria. Accessed 5th December, 2013 from http://www.dfid.gov.uk/news/files/success_stories/africa/nigeria_malaria.asp

Dillion WR, Goldstein M (1984). Multivariate analysis: Methods and applications. New York: John Wiley and Sons.

Hay SI, Myers MF, Burke M, Vaughn DW Endy T, Ananda N, Shanks GN, Snow GW, Rogers DJ (2002). Etiology of Inter-epidemic Periods of Mosquito – Borne Diseases. Proc. Natl. Acad. Sci. 97:9335–9339.

Idowu AP, Okoronkwo N, Adagunodo RE (2009). Spatial Predictive Model for Malaria in Nigeria. J. Health Inform. Dev. Ctries. 3(2):30-36.

Lindsay SW, Birley MH (1996). Climate Change and Malaria Transmission." Ann. Trop. Med. Parasitol. 90:573–588.

Lindsay SW, Martens WJM (1998): Mapping the Ranges and Relative Abundance of the two Principal African Malaria Vectors, *Anopheles gambiae* Sensustricto and *Anopheles arabiensis*, Using Climate Data*". Proc R. Soc Lond.* B. Biol. Sci. 265:847–854.

Martens WJM, Niessen LW, Rothmanss J, McMichael AJ (1995). "A Potential Impacts of Global Climate Change on Malaria Risk." Environ. Health Perspect. 103:458–464.

National Population Commission (NPC) [Nigeria], National Malaria Control Programme (NMCP) [Nigeria], and ICF International (2012). Nigeria Malaria Indicator Survey 2010. Abuja, Nigeria: NPC, NMCP, and ICF International.

Oluwafemi OA, Babatimehin OI,Oluwadare TS, Mahmud UM (2013). Mapping Malaria Case Event And Factors Of Vulnerability To Malaria In Ileife, Southwestern Nigeria: Using Gis. Ethiop. J. Environ. Stud. Manag. 6(4):2013

Shanks GD, SI Hay DI, Stern KB, Snow RW (2002). "Emerging Infections." Dis. 8:1404–1408.

Thompson A (2004). 'Malaria: When Politics Kills'. This Day Newspaper, March 23

United States Embassy in Nigeria (2011). Malaria fact Sheet. Accessed 5th December, 2013 from http://photos.state.gov/libraries/nigeria/231771/Public/December-MalariaFactSheet2.pdf

USAID (2011). NIGERIA FY 2012 Malaria Operational Plan Accessed 5th December, 2013 from http://pmi.gov/countries/mops/fy12/nigeria_mop_fy12.pdf

Votava A, Harlow J, Running S (2000). "Weather and Malaria Transmission: The Unfolding Drama." In Harlow J. Malaria and Modeling of East Africa.NumericalTerra – Dynamic Simulation Group, University of Montana, Missouri, USA.

World Health Organization (2000). "$1 to Roll Back Malaria." Partnership 1(8):9.

World Health Organization (2005). "WHO's Malaria Country Report." World Health Organization Statistical Information System (WHOSIS): Country Official Health Indicators. Geneva.

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