Niger. J. Physiol. Sci. 29(June 2014) 001 –005
www.njps.com.ng

Niger. J. Physiol. Sci.

Profile of children with new-born brachial plexus palsy managed in a tertiary hospital in Ibadan, Nigeria

*** Omoyemi O. Ogwumike[1], Ade F. Adeniyi[1], Umar Badaru[2], Joan O. Onimisi[1]**
Department of Physiotherapy, [1]College of Medicine, University of Ibadan, Ibadan, Nigeria
[2]Faculty of Medicine, Bayero University, Kano, Nigeria

Summary: New-born Brachial Plexus Palsy (NBPP) is birth injury resulting from traction to the brachial plexus at birth. It is an injury to two or more cervical or thoracic nerve roots. It has been reported to be one of the most common birth injuries of the new-born. Persistent presentation of children with NBPP in Nigeria paediatric practice necessitates the need to examine prevalence, patterns and possible predisposing factors. This study therefore investigated retrospectively, profiles of children who presented with NBPP over a ten year period and were managed at the Physiotherapy department of the University College Hospital, Ibadan Nigeria. A retrospective cross-sectional survey, in which files of children with NBPP located from the database of the Physiotherapy department were retrieved in order to assess infant and maternal information. One hundred and seventy children, 93 (54.7%) males and 77 (45.3%) females were studied. Their mean birth weight was 4.21±0.54 kg, 99 (58.2%) were delivered in private (non-government) hospitals, majority 163 (95.9%) presented with Erb's Palsy. Mothers, 54 (31.8%) were mostly primiparous and a seemingly persistent elevation in two-yearly incidence of NBPP was observed. Improved healthcare policy in child delivery is encouraged in non-government hospitals in Nigeria to provide preventive measures toward incidence of NBPP. Documentation of physiotherapy management of children with NBPP should include outcomes from admission through to discharge.

Keywords*:* New-born, Brachial plexus Palsy, Incidence, Physiotherapy, Nigeria

**Address for correspondence:* yemfide@yahoo.com

Manuscript Accepted: February 2014

INTRODUCTION

New-born brachial plexus palsy (NBPP) also known as neonatal brachial plexus palsy results from injury to two or more cervical and thoracic nerve roots (C5-T1) that occurs before, during or after the birth process (Foad et al, 2008). Waters (2005) defined NBPP as paralysis of the upper extremity secondary to a traction or compression injury to the brachial plexus sustained at birth.

The brachial plexus forms a network of nerves that conduct nerve impulses that control the muscles of the shoulder, arm, wrist, hand and fingers. The mechanism of NBPP is attributed to severe lateral flexion of the neonate's neck when the shoulder is stopped most often at the pubic bone during delivery. The extent of the traction on the brachial plexus therefore result in various injury types to the nerves ranging from a simple mild stretch up to an avulsion of the nerve roots from the spinal cord (Sutcliffe, 2007).

Incidence of NBPP as reported by Hoeksma et al, (2004) ranged from 0.38 to 5.1 per 1000 live births in Amsterdam, Netherlands. Waters (2005) also reported a frequency of 0.38 to 1.56 per 1000 deliveries. In the United States, a 3 year study of NBPP by Foad et al, (2008) reported an incidence of 1.51 ± 0.02 per 1000 live births with the rate decreasing over the study time period.

In the developing countries of the world, particularly in the sub-Saharan Africa investigating the incidence of NBPP is essential in view of persistent occurrence of this problem. In addition, few studies have been conducted in Nigeria on NBPP. Ogunlade et al (2005) in a study of skeletal birth injuries reported prevalence of NBPP to be 66.7% within a four year study period, Oluwadiya et al, (2005) reported 43.8% and Hamzat et al (2008) in a study of prevalence of NBPP in Accra Ghana over a five year period reported 27.2% prevalence. Various clinical factors have been proposed to be associated with occurrence of NBPP including prolonged labour, maternal diabetes, assisted delivery, breech presentation, macrosomia and high maternal body mass, high birth weight, place of birth, antenatal practices and a host of others (Narchi et al, 1996; Berard et al, 1998; Raio et al, 2003; Pondaag et al, 2004; Hamzat et al, 2008; Onalo et al, 2011).

This present study was undertaken to retrospectively investigate profiles of children who

presented with NBPP in a tertiary hospital in Ibadan Nigeria and examine prevalence in incidence, patterns and factors associated with occurrence of NBPP.

MATERIALS AND METHODS

Ethical approval for this study was obtained from the University of Ibadan/University College research ethics committee (UI/EC/12/0116). The study was a retrospective cross-sectional study. It involved cases of children with NBPP managed at the physiotherapy department of the University College hospital, Ibadan Nigeria over a ten year period (January 2002 to December 2011). The University College hospital, Ibadan Nigeria is a specialist tertiary hospital which is a major referral centre in the South-West sub-region of Nigeria from different parts of the country particularly from primary healthcare centres, non-government/private healthcare facilities, and secondary healthcare facilities in various wards, local government areas and states of the Nigerian federation.

Children with NBPP diagnosed by paediatricians, paediatric neurologists or orthopaedic surgeons are usually referred to the paediatric section of the physiotherapy department for further assessment and physiotherapy management. For the purpose of this study, the database of the department of physiotherapy was searched to identify cases of paediatric conditions including NBPP managed over the above-mentioned years in order to locate the files. The files of children managed for NBPP were identified and separated to assess relevant information on child and maternal characteristics. These include age of child, age at presentation for physiotherapy management, year of presentation, place of delivery of child, type of delivery, birth presentation, type of NBPP, events at delivery, maternal occupation, parity of mother, outcomes of physiotherapy management of child and discharge. Classification of the type of NBPP is usually done using the Narakas system (Narakas, 1987) in which injury to C5 and C6 plus or minus C7 is described as upper brachial plexus injury or Erb's palsy, injury to the above roots along with C8 and T1 is referred to as total palsy with no Horner's syndrome-here a functional hand may be seen in many patients, an injury to C5, C6, C7, C8 and T1 with Horner's syndrome is described as complete flaccid paralysis or flail arm (Narakas, 1987).

Physiotherapy management usually begins after a careful history taking and detailed clinical examination, checking for possible associated injuries like fractures of the clavicle or humerus (Thatte and Mehta, 2011). The treatment plan is usually aimed at maintaining the physiological state of the affected upper limb by prevention of muscle shortening and stiffness of the affected joints, restoration of motor function and prevention of muscle atrophy. The above are achieved by gentle passive mobilisation of all joints of the affected limb along with soft tissue mobilization. Mothers are taught how to carry out these movements to the affected upper limb of the child to make for continuity of treatment and adequate frequency as required in the home (Sutcliffe, 2007). In cases of severe forms of NBPP, mild intensity of neuromuscular electrical stimulation (NMES) usually accompanies the above protocol and this help to prevent muscle atrophy while the nerves regain their function (Nath et al, 2010).

A limitation in this study however, was that some variables were missing from some files. This therefore reduced the available information on some child and maternal characteristics and this were noted as not recorded.

Statistical analysis:
The data obtained were entered into a spread sheet after which it was analysed using descriptive statistics of mean, percentages and charts.

RESULTS

Characteristics of Children with New-born Brachial Plexus Palsy

One hundred and seventy cases of NBPP were identified within the study period (Jan 2002 to Dec 2011). Over half 93 (54.7%) were males while 77 (45.3%) were females. The greatest percentage of patients 87 (51.2%) were presented for physiotherapy management within the first four weeks of life. Of the 158 (92.9%) of children who had their birth weights recorded, 102 (60%) were within the range (4.0-6.5) kg. Least birth weight was 2.5 kg and the overall mean birth weight of infants was 4.21±0.54 kg. Majority of the children 99 (58.2%) were delivered in non-government hospitals (private hospital facilities). Table 1 shows the socio-demographic profile of these children.

Out of 135 (79.4%) children with NBPP, who had their mode of delivery recorded, 91 (53.5%) were delivered with assistance while 21 (12.4%) were through spontaneous vertex delivery. In terms of types of NBPP, Erb's palsy was the most presented by 164 (96.5%) of the patients while complications such as birth asphyxia 11 (6.4%) and humeral and clavicular fractures 1 (0.6%) and 2 (1.2 %) respectively were associated problems in some of the children.

Outcome of physiotherapy management was not readily available as only 2 (1.2 %) of the patients had records of being formally discharged in their case files. This can be observed in Table 2.

Maternal Characteristics of Children with New-born Brachial Plexus Palsy

In 166 (97.6%) cases maternal age was not recorded, majority of the mothers 156 (91.7%) were married,

and employed 131 (77.1%) and mostly primiparous 54 (31.8%). The commonest event at delivery for most of them 95 (55.8%) is prolonged labour. This is as seen in Table 3.

Table1. Socio-demographic Profile of Children with Newborn Brachial Plexus Palsy

Variables	N	%
Sex		
Male	93	54.7
Female	77	45.3
Age at presentation for Physiotherapy (weeks)		
0.0-4.00	87	51.2
4.10-8.00	31	18.2
8.10-12.00	17	10
12.10-16.00	2	1.2
>16.00	18	10.6
Not recorded	15	8.8
Birth weight		
2.50-2.99	4	2.4
3.00-3.49	8	4.7
3.50-3.99	44	25.9
4.00-4.49	64	37.6
4.50-4.99	30	17.6
>5.0-6.50	8	4.7
Not recorded	12	7.1
Place of birth		
Private Hospitals	99	58.2
Government hospitals	17	10.0
Mosques/Churches/Mission	12	7.1
Homes	3	1.8
Not recorded	39	22.9

Table 2: Clinical Profile of Children with Newborn Brachial Plexus Palsy

Variables	N	**%**
Mode of delivery		
Spontaneous vertex	21	12.4
Assisted (forceps, vacuum extraction)	91	53.5
Cesarean section	23	13.5
Not recorded	35	20.6
Type of NBPP		
Erb's palsy	164	96.5
Total BPP	6	3.5
Associated problems		
Birth asphyxia	11	6.4
Humeral fracture	1	0.6
Clavicular fracture	2	1.2
Shoulder dislocation	1	0.6
None	155	91.2
Discharge/Outcome of physiotherapy		
Discharged	2	1.2
Being considered for discharge	2	1.2
Referred	4	2.4
Nil record of discharge	162	95.2

Key: NBPP- Newborn brachial plexus palsy.

Prevalence and Incidence of NB*PP over the ten year study period*

Prevalence of children with NBPP over the ten year study period shows a persistent high prevalence in the years 2007, 2008 and 2009 (17.1%, 18.1% and 15.3%) respectively. The least prevalence (5.4%) was observed in year 2002. In figure 1, a two-yearly incidence of NBPP is illustrated. Persistent high incidences (26.5%, 25.3% and 20.5%) occurred in years 2006-2007, 2008-2009 and 2010-2011 respectively, while the least (8.8%) occurred in years 2002-2003.

Table 3: Maternal Characteristics of Children with Newborn Brachial Plexus Palsy

Variables	N	%
Age of Mother (yrs)		
28-35	4	2.4
Not recorded	166	97.6
Marital Status		
Married	156	91.7
Not Married	3	1.8
Not recorded	11	6.5
Occupation		
Unemployed	24	14.1
Employed	131	77.1
Not recorded	15	8.8
Parity		
1	54	31.8
2	40	23.5
3	34	20
4	17	10
>5	11	6.5
Not recorded	14	8.2
Events at delivery		
Prolonged labour	95	55.9
Caesarean section	23	13.5
Episiotomy, perineal tears	17	10.0
Not recorded	35	20.6

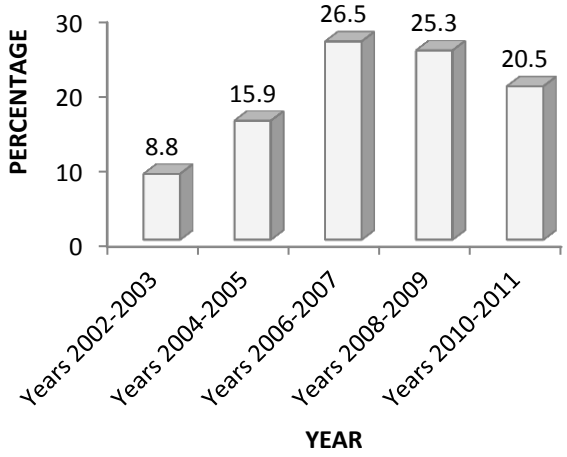

Figure 1: Two-yearly incidence of Newborn Brachial Plexus Palsy in the University College Hospital Ibadan over a ten year period

DISCUSSION

This study investigated profiles of children with NBPP managed in the physiotherapy department of the University College Hospital Ibadan, Nigeria. The age at presentation was mostly in the range (0-4) weeks, revealing early presentation for physiotherapy treatment. Indeed it has been observed that early initiation of physiotherapy for children with NBPP after birth is essential for early functional recovery in the upper limb. Others who presented later, (>4-12) weeks after birth could have done so due to a delay in diagnosis of NBPP and subsequent delay in referral for physiotherapy (Oluwadiya et al, 2005). Notable in this instance, particularly are children with NBPP who were delivered in some private hospital facilities. These hospitals may sometimes lack adequate experienced manpower and diagnostic skills. Such practices therefore may be unable to explain the cause of occurrence of NBPP to the mother of the affected child. The consequent inability to make early referral may cause a delay in the much needed and appropriate intervention for the child.

Mode of delivery of most of the children with NBPP was through assisted means (forceps, vacuum extraction). These are usually difficult deliveries and even those children who were delivered through Caesarean section in this study could also have had NBPP due to hard labour and attempted delivery before Caesarean section proved to be necessary.

High birth weights have been observed to be a remarkable etiologic factor for NBPP (Piatt, 2004; Sutclifffe, 2007; Ruchelsman et al, 2009). In this study, children with high birth weights formed the highest percentage of those with NBPP. It is noteworthy that maternal gestational diabetes has been attributed to high birth weights (Piatt, 2004) even though in this study, no record of maternal gestational diabetes was made probably because the assessment in the case files were essentially that of the child with NBPP.

Of the different forms of NBPP, Erb's palsy was mostly observed. This observation is similar to that of Ruchelsman et al, (2009). A probable explanation for this has been attributed to the upper trunk roots of the brachial plexus (C5 and C6) being more susceptible to traction force compared to the lower trunk roots (C8 and T1).

Of the few associated injuries or problems with NBPP in this study, birth asphyxia had highest frequency of observation. This probably was due to prolonged labour, which in turn could have been responsible for oxygen deprivation of the infant. Protracted labour especially at the second stage of labour has been observed as a risk factor for NBPP (Sutcliffe, 2007).

A consideration of outcomes in physiotherapy management of children with NBPP revealed that largest percentage had no discharge records while only 1.2% was reported discharged. This inadequate documentation of treatment outcome could be due to a variety of reasons: firstly as management of this condition is a relatively long term procedure, parents could easily default from coming for treatment on observation of a measurable functional recovery in the upper limb of their children. Secondly distractions due to day to day stress of making a living coupled with economic hardship could have discouraged the parents from bringing the children for treatment until full recovery is achievable. Thirdly the attending physiotherapist could have overlooked the importance of periodic review of the patient management and documenting recommendations for discharge when necessary ultimately resulting in omission of documentation of discharge when there is complete functional recovery of the upper limb of the patient.

In this study, primiparous women had the highest frequency of children with NBPP. This observation is in agreement with that of Tandon and Tandon, (2005) in which the observation was made that brachial plexus injury is more likely to occur in neonates from primiparous women especially in the presence of shoulder dystocia. Mothers who had prolonged labour before birth were also more likely to have a child with NBPP. In fact the observation made in a study by Al-Rekabi (2011) in Bint Al-Huda Teaching Hospital, Thi-Qar revealed that prolonged labour is a risk factor for NBPP.

Findings in this study show that prevalence of NBPP among the paediatric population who presented at the physiotherapy department, University College Hospital constituted a significant proportion of cases seen. There also appeared to be a persistent elevation in two-yearly incidence as well as prevalence of NBPP within the ten-year study period.

Conclusion

Erb's palsy is one form of NBPP most frequently managed in the paediatric unit of the physiotherapy department, University College Hospital Ibadan. Improved healthcare policy in child delivery should be encouraged, particularly in non-government facilities where most children with NBPP are reportedly delivered. During management of patients with NBPP, adequate documentation by attending physiotherapists is recommended in order to provide records on outcome/discharge; this may in turn provide essential data to estimate prognostication of NBPP.

Acknowledgement

We would like to thank Mrs S.O Awoyele and other members of staff of the records unit, Department of Physiotherapy, University College Hospital Ibadan for their selfless effort in assisting in retrieving the files of the children involved in this study

REFERENCES

Al-Rekabi, AM 2011 Brachial Plexus Palsy among Neonates in Bint Al-Huda Teaching Hospital Thi-Qar. *Thi-Qar Medical Journal* 5 (3): 50-55.

Bérard J, Dufour P, Vinatier D, et al 1998 Fetal macrosomia: risk factors and outcome. *Eur J Obstet Gynecol Reprod Biol* 77(1): 51-59.

Ecker JL, Greenberg JA, Norwitz ER, et al 1997 Birth weight as a predictor of brachial plexus injury. *Obstet Gynecol* 89 (5 pt 1) 643-7.

Foad SL, Mehlman CT, Ying J 2008. The epidemiology of Neonatal Brachial Plexus Palsy in the United States. *J Bone Joint Surg* 90: 1258-1264.

Hamzat TK, Carsamer S, Wiredu EK 2008. Prevalence of new-born brachial plexus palsy in Accra, Ghana. *J Pediatr Neurol* 6:133-138.

Hoeksma A, Tersteeg AM, Neilssen RGH 2004. Neurological recovery in obstetric brachial plexus injuries: An historical cohort study. *Dev Med Child Neurol* 46: 76-83.

Narchi, H, Kulaylat, N, Ekuma-Nkama, E 1996 Clavicle Fracture and Brachial Plexus Palsy in the Newborn: Risk Factors and Outcome. *Ann Saudi Med* 16 (6):707-710.

Narakas, AO 1987. Obstetric brachial plexus injuries. In: Lamb DW (ed). *The paralysed hand*. Edinburgh: Churchill Livingstone, pp. 116-35.

Nath RK, Karicherla P, Mahmooduddlin F 2010 Shoulder function and anatomy in complete obstetric brachial plexus palsy: long-term improvement after triangle-tilt surgery. *Child Nerv Syst* 26: 10009-19.

Ogunlade SO, Omololu AB, Alonge TO et al, Skeletal and birth injuries: presentation, management and outcome at the University College Hospital, Ibadan. Nigeria. *Niger J Pediatr* 32:12.

Oluwadiya KS, Olasinde, AA, Ukpai OB et al 2005 Birth injury: a retrospective study of 146 cases in three Teaching Hospitals in Nigeria. *Internet J Pediatr Neonatol* 5 (2).

Onalo R., Ogala WN, Ameh N, et al, 2011 Perinatal Presentation and Outcome of High Birth weight Infants in Zaria, Nigeria. *Niger J Paediatr* 38(2):65-72.

Piatt, JH 2004 Birth Injuries of the Brachial Plexus. *Paediatr Clin N Am* 51, 421-440.

Pondaag W, Malessy, MJA, Gert van Dijk, J., et al, 2004 Natural History of Obstetric Brachial Plexus Palsy: A Systematic Review. *Dev Med Child Neurol* 46 (2), 138-144.

Raio L, Ghezzi F, Di Naro E, et al. 2003 Perinatal outcome of fetuses with a birth weight greater than 4500 g: an analysis of 3356 cases *Eur J Obstet Gynecol Reprod Biol* 109(2):160-5.

Ruchelsman, DE, Pettrone S, Price AE, et al, (2009) Brachial Plexus Birth Palsy. An Overview of Early Treatment Considerations. *Bull NYU Hosp Joint Dis* 67(1):83-9.

Sutcliffe TL. Brachial plexus injury in the newborn 2007. *NeoReviews (American Academy of Paediatrics)* 8: e239-e246.

Tandon S, Tandon V, 2005 Primiparity: a risk factor for brachial plexus injury in the presence of shoulder dystocia. *J Obstet Gynaecol* 25(5):465-8.

Thatte MR, Mehta R, 2011 Obstetric brachial plexus injury. *Indian J Plastic Surg* 44 (3): 380-389.

Waters PM. 2005 Update on management of pediatric brachial plexus palsy. *J Pediatr Orthop* 14A: 233-44.

Niger. J. Physiol. Sci. 29(June 2014) 011 –015
www.njps.com.ng

Niger. J. Physiol. Sci.

Effect of zinc treatment on intestinal motility in experimentally induced diarrhea in rats

*** Adeniyi O. S.[1], Akomolafe R. O.[2], Ojabo C.O.[3], Eru E.U. [1] and Olaleye S.B.[4]**

[1]Department of Physiology, College of Health Sciences, Benue State University, Makurdi, Nigeria, [2]Department of Physiological Sciences, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria, [3]Department of Ophtalmology, College of Health Sciences, Benue State University, Makurdi, Nigeria, [4]Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Summary: Zinc supplementation is a critical new intervention for treating diarrheal episodes in children. Recent studies suggest that administration of zinc along with new low osmolarity oral rehydration solutions / salts (ORS) can reduce the duration and severity of diarrheal episodes for up to three months. Several mechanisms of action of zinc has been proposed, however there is dearth of information about the effect of zinc on intestinal motility during diarrhea. Male albino Wistar rats (80-100g) were used. The effect of different doses of zinc sulphate (25, 50, 100, 150mg/Kg) on the number of wet faeces was investigated. Intestinal motility during castor oil induced diarrhea was assessed using activated charcoal meal and the mechanisms of action of zinc sulphate on motility were investigated. The effective dose of zinc sulphate (100mg/Kg) significantly reduced ($p< 0.001$) the number of wet faeces (3.0 ± 0.00) compared with control (6.8 ± 0.25) during diarrhea. This antidiarrheal effect of zinc was abolished by propranolol and nifedipine. Zinc sulphate significantly reduced ($p< 0.05$) intestinal transit time (60.7 ± 7.13%) compared with control (85.7 ± 2.35%). It is concluded that zinc sulphate reduces the frequency of wet faeces output and intestinal motility during diarrhea via activation of β adrenergic receptor and L-type Ca^{2+} channel.

Keywords*:* Diarrhea, Zinc, Intestinal motility, Adrenergic receptor, Calcium channel.

**Address for correspondence:* supoadeniyi@yahoo.com Phone: +2348051980450

Manuscript Accepted: April, 2014

INTRODUCTION

Diarrhea is the passage of three or more unformed stool per day, often in addition to other enteric symptoms, or the passage of more than 250 g of unformed stool per day (DuPont 2004). It includes loss of electrolytes and water and decreased absorption of fluid from the gastrointestinal tract. It also involves increased frequency of bowel movements (Lakshminarayana *et al*., 2011). Diarrhea has long been recognized as one of the most important health problems and leading cause of mortality and morbidity in the developing countries (Rajamanickam *et al*., 2010) and produces more illness and causes death of more infants and children below 5 years old than all other diseases combined (Dalal *et al*., 2011). Diarrhea is considered as one of the leading causes of growth retardation and death in infants (Petri *et al*., 2008).

Acute diarrhea remains a leading cause of childhood deaths despite the undeniable success of Oral Rehydration Therapy (ORT). Oral rehydration solution (ORS) saves children's lives, but does not seem to have any effect on the length of time the children suffer with diarrhea. Hence, new revised recommendations have been formulated by the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF), in collaboration with the United States Agency for International Development (USAID) and other experts. It recommends zinc salt along with low osmolarity ORS, with reduced levels of glucose and salt, during acute diarrhea, which reduced the duration and severity of the episode; and zinc supplementation given for 10-14 days lowers the incidence of diarrhea in the following two to three months (WHO / UNICEF Joint Statement, 2004).

Zinc is an essential micronutrient for human growth, development, and maintenance of the immune system. Zinc supplementation has been found to reduce the duration and severity of diarrheal episodes and likelihood of subsequent infections for 2–3 months (Bhutta *et al*., 2000). Zinc supplements are generally accepted by both children and caregivers and are effective regardless of the type of common zinc salt used (zinc sulphate, zinc acetate or zinc gluconate). The positive action of zinc in acute diarrhea are due to the ability of zinc to improve absorption of fluid and electrolyte, improve regeneration of the intestinal epithelium, increase the levels of brush border enzymes, increase protein synthesis, cell growth and differentiation, improve immune function, and regulate oxidative stress (Berni *et al*., 2011; Patel *et al*., 2005). Zinc inhibits toxin-

induced cholera, but not Escherichia coli heat-stable, enterotoxin-induced, ion secretion in cultured Caco-2 cells (Berni *et al*., 2005).

Literature review however revealed dearth of information about the effect of zinc on intestinal motility during diarrhea. Alterations in intestinal motility (usually increased propulsion) are observed in many types of diarrhea. This research was therefore carried out to investigate the effect of zinc on intestinal motility during diarrhea and the mechanism by which zinc might affects motility.

MATERIALS AND METHODS

Male albino Wistar rats (80-100g) were used for the experiments. The animals were housed under standard controlled environmental conditions with a 12 hour light/dark cycle, with food (Pfizer Feed Plc, Nigeria) and water provided *ad libitum*. The animals were allowed to acclimatize for one week before the experiments.

The rats were then kept in plastic cages whose floors were lined with white blotting paper for two hour daily in order to allow them to be familiar with the environment two weeks. Wire gauze was placed about 2 cm above the papers so as to prevent the rats from eating up the papers. For all the experiments, the animals were fasted for 18 hours before treatment.

Effects of zinc on fecal pellet output in castor oil induced diarrhea

The antidiarrheal activity of zinc sulphate was evaluated according to the method described by Teke *et al*. (2007). The animals were fasted for 18 hours and divided into five groups of five animals each. All rats were put in a separate cage. Each animal was given 1ml castor oil. After thirty minutes, each animal in the different groups were treated as follows: group 1 was administered 10ml/Kg normal saline (p.o); group 2, loperamide (3mg/Kg, p.o); group 3, zinc sulphate (25mg/Kg, p.o); group 4, zinc sulphate (50mg/Kg, p.o); group 5, zinc sulphate (100mg/Kg, p.o) and group 6, zinc sulphate (150 mg/Kg, p.o). The fecal pellets of the rat was counted every 2 hours from the time of zinc treatment for the first 8 hours and weight of faeces overnight (24 hours) after diarrhea induction were obtained. The presence of wet faeces was noted for each animal. The pellets were air dried for another 24 hours before weighing. The average weight of the pellets was taken as the output for each rat in the group.

Effects of blockers on fecal pellet output in castor oil treated animals given zinc.

The dose that produced the greatest effect on fecal pellet output was used as the working dose for this study. Twenty-five rats were divided into five groups of 5 rats each. Each rat in group 1 was pre-treated with 10ml/Kg normal saline (i.p.), while each rat in group 2 was given 10 mg/kg propranolol (i.p), group 3, 1mg/Kg prazosine (i.p), group 4 was given 2.5mg/Kg nifedipine (i.p) and group 5 was given 0.1mg/Kg atropine (i.p). Thirty minutes later, each animal was given 1ml of castor oil and after 30 minutes, each rat was given the working dose of zinc. Fecal pellet output was recorded every hour for 4 hours.

Effects of zinc on intestinal transit

The method of Gamaliel *et al*., (1996) was used. Fifteen rats were divided into three groups of 5 rats each. Each animal was given 1ml of castor oil. Thirty minutes after castor oil administration, animals in group 1 were given 3ml/Kg normal saline (p.o), animals in group 2 were administered 0.8ml of working dose of zinc and animals in group 3 were given 0.1mg/kg standard drug atropine (i.p). Thirty minutes after the last dose of drug was given 10% activated charcoal in physiological saline in a volume of 3ml/Kg was administered to each animal. One hour after charcoal meal, the rats were sacrificed and the abdomen immediately cut open to dissect out the whole small intestine (pylorus region to caecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured for obtaining the percentage of the entire small intestine travelled by the activated charcoal as described below:

$$\%\ \text{distance travelled} = \frac{\text{distance travelled}}{\text{Total length of the intestine}} \times 100$$

Statistical analysis:

Results were presented as mean ± standard error of mean (SEM). The student t-test was used to determine the significant difference between two groups. Confidence interval of 95% was taken as statistically significant. Data was analysed using SPSS version 17 software.

RESULTS

Effects of zinc on wet fecal pellet output

Result showed that castor oil administration induced diarrhea (wet faeces) in the animals. Zinc sulphate administration reduced the number of faecal pellet output. This inhibition was not effective at 25mg/Kg Zinc sulphate administration ($p>0.05$). However, zinc sulphate at a dose of 50mg/Kg, 100mg/Kg and 150mg/Kg significantly ($p<0.05$) reduced the number of wet feacal pellets compared with control. Table 1 showed that 8 hours after rats were given castor oil, the mean number of wet faeces in control rats was 6.8 ± 0.25. Zinc sulphate (50mg/Kg) significantly ($p< 0.05$) reduced the number of wet faeces (5.0 ± 0.41), a dose of 150mg/Kg significantly ($p< 0.001$) reduced wet fecal pellet output (3.3 ± 0.25) and the most effective dose in this study was 100mg/Kg which significantly ($p< 0.001$) reduced the number of wet faeces to 3.0 ± 0.0. The 100mg/Kg dose was therefore

taken as the Working Dose. The standard antidiarrhea drug loperamide (3mg/Kg) produced a significant reduction ($p< 0.001$) in number of wet faeces output all through the 8 hours of study. The reduction in number of wet output in loperamide treated animals was not significantly different from that in zinc treated (100mg/Kg)

Effects on zinc on mean weight of faeces after 24 hours castor oil induced diarrhea

Figure 1 showed that all doses of zinc sulphate used in this study (25, 50, 100, 150mg/Kg) produced significant ($p< 0.05$) decrease in the mean weight of faeces after 24 hours of diarrhea induction and drug treatment. Loperamide (3mg/Kg) also produced a decrease in faecal weight after 24 hours of treatment as shown in Figure 1.

Effects of blockers on wet faecal pellet output in castor oil treated animals given zinc

Result showed that by the 4th hour after diarrhea was induced, propranolol and nifedipine significantly ($p< 0.001$, 0.001 respectively) abolished the antidiarrhea effect of zinc sulphate. There was no significant difference ($p> 0.05$) in number of wet faeces between prazosine treated animals and animals given normal saline, while the antidiarrheal effect of zinc sulphate was further potentiated by atropine. There was no wet faeces in animals treated with atropine. (Table 2)

Effects of blockers on mean weight of faeces after 24 hours

Figure 2 showed that propranolol and nifedipine increased the mean faecal weight when administered to rats before zinc sulphate treatment after diarrhea has been induced in rats; however this increase were not significant ($p> 0.05$). Prazosine decreased the mean weight of faeces of rats; however this decrease was not significant ($p> 0.05$). The mean weight of faeces was significantly reduced by atropine ($p< 0.05$).

Table 1. Effects of Zinc treatment on number wet feacal output in castor oil treated rats

Treatment	2 hours	4 hours	6 hours	8 hours
Control	3.8 ± 0.25	5.5 ± 0.29	6.5 ± 0.29	6.8 ± 0.25
3mg/Kg Loperamide	2.3 ± 0.25**	2.3 ± 0.25**	2.5 ± 0.29***	2.5 ± 0.29***
25mg/Kg Zinc sulphate	4.0 ± 0.41	5.3 ± 0.25	6.3 ± 0.26	6.5 ± 0.29
50mg/Kg Zinc sulphate	3.5 ± 0.29	4.3 ± 0.25*	5.0 ± 0.41*	5.0 ± 0.41*
100mg/Kg Zinc sulphate	3.0 ± 0.0*	3.0 ± 0.0***	3.0 ± 0.0***	3.0 ± 0.0***
150 mg/Kg Zinc sulphate	2.5 ± 0.29*	3.3 ± 0.25**	3.3 ± 0.25***	3.3 ± 0.25***

N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$, **= significant compared with control at $p< 0.01$, *** = significant compared with control at $p< 0.001$

Table 2: Effect of blockers on number of wet faeces in castor oil treated animals given zinc.

Treatment	1 hours	2 hours	3 hours	4 hours
Control	0.5 ± 0.29	1.8 ± 0.25	2.5 ± 0.29	3.0 ± 0.41
Propranolol	3.3 ± 0.25***	4.8 ± 0.25***	5.5 ± 0.29***	5.8 ± 0.25***
Prazosine	2.0 ± 0.0**	3.0 ± 0.0**	3.8 ± 0.25*	3.8 ± 0.25
Nifedipine	2.8 ± 0.25**	4.8 ± 0.25***	5.5 ± 0.29***	6.0 ± 0.0***
Atropine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$, **= significant compared with control at $p< 0.01$, ***= significant compared with control at $p< 0.001$

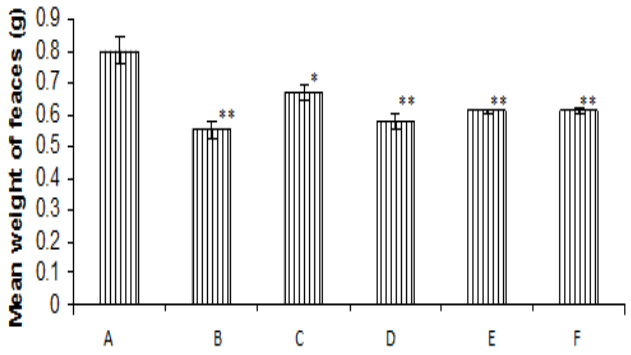

Figure 1: Mean weight of faecal output after 24 hours of castor oil induced diarrhea. N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$, **= significant compared with control at $p< 0.01$, *** = significant compared with control at $p< 0.001$. A=control, B=3mg/Kg Loperamide, C=25mg/Kg $ZnSO_4$, D=50mg/Kg $ZnSO_4$, E=100mg/Kg $ZnSO_4$, F=150 mg/Kg $ZnSO_4$

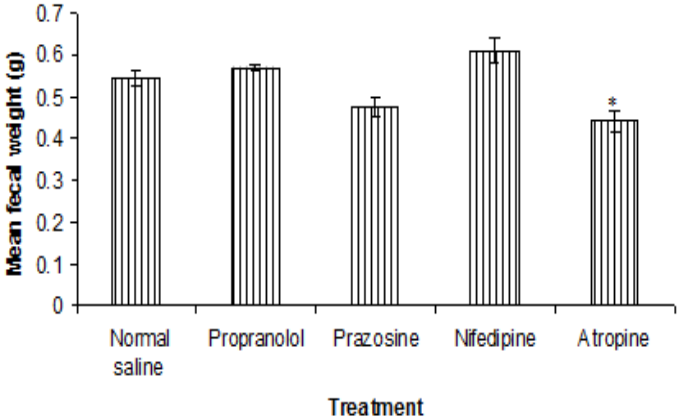

Figure 2: Effect of blockers on mean faecal weight after 24 hours. N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$.

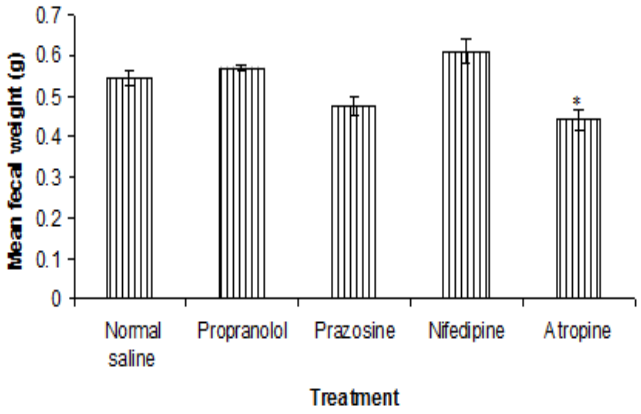

Figure 3: Percentage distance moved by charcoal meal through the small intestine. N= 5, values are presented as mean ± SEM. *= significant compared with control at p< 0.05, **= significant compared with control at p< 0.01

Effects of zinc on intestinal transit

In control animals given normal saline, charcoal meal moved 85.7 ± 2.35% through the length of the small intestine. Zinc sulphate treatment (100mg/Kg) significantly (p< 0.05) reduced the distance moved by charcoal (60.7 ± 7.13%), while the standard drug atropine significantly (p< 0.01) reduced distance moved by charcoal (65.9 ± 4.08%) as shown in Figure 3.

DISCUSSION

The result of this study agrees with previous reports on the effect of zinc on diarrhea and in addition to earlier mechanisms of action elucidated on zinc in its antidiarrhea activities, this study showed that zinc treatment reduced intestinal motility during castor oil induced diarrhea. Zinc sulphate was not very effective at the lower doses of 25mg/Kg and 50mg/Kg. However, zinc was more effective at higher doses and a dose of 100mg/Kg was more effective than 150mg/Kg. The induction of diarrhea with castor oil results from its hydrolytic product, ricinoleic acid, (Iwao and Terada, 1962). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion, (Pierce *et al.*, 1971) resulting in the generation of giant contractions of the transverse and distal colon (Croci *et al*., 1997). A potential antidiarrheal agent may exhibit its antidiarrheal effect by inhibiting either gut motility and/or electrolyte out flux (Croci *et al.*, 1997), therefore zinc sulphate is an important antidiarrheal agent.

The result of this study revealed that the antidiarrheal effect of zinc occur by stimulation of β-adrenergic receptors in the intestine. Gati *et al*., (1975) had previously suggested that epinephrine exerts its inhibitory effect on gastric motility via beta adrenergic receptors, thus activation of the sympathetic innervations of the intestines results in the inhibition of peristaltic activity and a reduction in tone. Akomolafe *et al*., (2004) reported that both alpha and beta receptor stimulation are involved in antidiarrhea mechanism. Mohammed *et al*., (2009) also reported that the hydro methanolic portion of *Indigofera pulchra* extract possessed antidiarreal effect and that it acted through β adrenergic receptors. Therefore the adrenergic pathway is important in antidiarrhea mechanism. Many antidiarrhea drugs also exert their effects through α2-adrenoceptors pathway (Hsu, 1982; Ruwart *et al*., 1980). Activation of the pre-junctional α2-adrenoceptors on the parasympathetic terminals plays an important role in the inhibitory action of sympathetic nerve stimulation of gastrointestinal motility by inhibiting acetylcholine release (Berthelsen & Pettinger, 1977). The sympathetic nervous system also controls the balance between absorption and secretion in the ileum through activation of the mucosal α2-adrenoceptors.

This study also revealed that zinc sulphate reduced intestinal motility during diarrhea by a mechanism involving L-type Ca (2+) channels. Nifedipine, a Ca^{2+} blocker abolished the antidiarrheal effect of Zinc. This is in line with previous work done on antidiarrheal drug (Borrelli *et al*., 2006). Contractile activity in smooth muscle is initiated by a Ca^{2+}-calmodulin interaction to stimulate phosphorylation of the light chain of myosin, enabling the molecular interaction of myosin with actin thus causing contraction.

Zinc sulphate, acetate, and gluconate are all acceptable zinc salt formulations, of which zinc sulfate is low-cost, efficacious and safe. Zinc sulphate tablets may be dispersed in breast milk, in oral rehydration solutions, or in water on a small spoon; older children may chew the tablets or swallow them with water (Bajait and Thawani, 2011). Thus oral zinc administration provides substantial benefit in the reduction of stool output, frequency, and duration, combined with safety, efficacy, and affordability in acute diarrhea. This study reveals that zinc sulphate in addition to the initial mechanisms of action elucidated, reduces intestinal motility by activating β-adrenergic receptors and L-type Ca (2+) channel.

REFERENCES

Akomolafe R. O., Adeoshun I. O., Ayoka A. O., Elujoba A. A., Iwalewa E. O. (2004). An in vitro study of the effects of Cassia podocarpa fruit on the intestinal motility of rats. Phytomedicine. 11(2-3):249-54.

Bajait C. and Thawani V. (2011). Role of zinc in pediatric diarrhea. Indian J Pharmacol; 43(3): 232–235

Berni C. R., Buccigrossi V., Passariello, A (2011). Mechanisms of action of zinc in acute diarrhea. Current Opinion in Gastroenterol: 27 (1) 8–12.

Berni C. R., Cirillo P., Buccigrossi V., Ruotolo S., Annalisa P., De Luca P., Porcaro F., De Marco G. Guarino A. (2005). Zinc inhibits cholera toxin induced, but not Escherichia coli heat stable enterotoxininduced, ion secretion in human enterocytes. J Infect Dis;191:1072–7.

Berthelsen S. and Pettinger W. A. (1977): A functional basis for the classification of α2 adrenergic receptors. Life Sci 21: 595–606.

Bhutta Z. A., Bird S. M., Black R. E., Brown K. H., Hidayat A., Khatun F., Martorell R., Ninh N. X., penny M. E., Rosado J. L., Roy S. K., Ruel M., Sazawal S., Shankar A. (2000). Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. American Journal of Clinical Nutrition, 72(6):1516–22.

Borrelli F., Capasso F., Capasso R., Ascione V., Aviello G., Longo R., Izzo A. A. (2006). Effect of Boswellia serrata on intestinal motility in rodents: inhibition of diarrhoea without constipation. Br J Pharmacol. 148(4):553-60.

Croci M., Landi X., Emonds-Alt G., Le Fur J. P., Maffrand and Manara L. (1997). "Role of tachykinins in castor oil diarrhoea in rats," British Journal of Pharmacology, vol. 121, no. 3, pp. 375–380.

Dalal K., Singhroha S., Ahlawat S., Patra A. (2011). Anti-diarrhoeal activity of roots of Cicer arietinum Linn, Int. J. Res. Pharm. Bio Sci. 2(1): 268-270.

DuPont H.L. (2014). Acute infectious diarrhea in immunocompetent adults. N Engl J Med;370: 1532 – 1540.

Gamaliel K. S. and Akah P. A.. (1996). Analysis of the gastrointestinal relaxing effects of the stem bark extract of *Gongronema latifolium*. Phytomed., **2**: 293-296.

Gati T., Gelencser F., Hideg J. (1975). The role of adrenergic receptors in the regulation of gastric motility in the rat. Z Exp Chir.;8(3):179-84.

Hsu W.H., (1982). Xylazine induced delay of small intestinal transit in mice. European Journal of Pharmacology 83, 55–60.

Iwao I. and Terada Y. (1962) "On the mechanism of diarrhea due to castor oil," Japanese Journal of Pharmacology, vol. 12, pp. 137–145. View at ScopusT.

Lakshminarayana M., Shivkumar H., Rimaben P., Bhargava V.K. (2011). Antidiarrhoeal activity of leaf extract of moringa oleifera in experimentally induced diarrhea in rats. International journal of Phytomedicine;3:68-74.

Mohammed A., Ahmed H., Goji A. D. T., Okpanachi A. O., Ezekiel I. and Tanko Y. (2009). Preliminary Anti-diarrhoeal Activity of Hydromethanolic Extract of Aerial Part of *Indigofera Pulchra*.in Rodents. Asian Journal of Medical Sciences 1(2): 22-25.

Patel A. B., Dhande L. A., Rawat M. S. (2005). Therapeutic evaluation of zinc and copper supplementation in acute diarrhea in children: double blind randomized trial. *Indian Pediatrics*, 42(5):433–42.

Petri W. A., Miller M., Binder H. J., Levine M. M., Dillingham Rebecca., Guerrant R. L. (2008). Enteric infections, diarrhoea, and their impact on function and development, J. Clin. Invest; 118: 1277–1290.

Pierce N. F., Carpenter C. C .J., Elliot H. Z. and Greenough W. B. (1971). Effects of prostaglandins, theophylline and Cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum, Gastroenterol, 60: 22-32.

Rajamanickam V., Rajasekaran A., Anandarajagopal K., Sridharan D., Selvakumar K., Rathinaraj S. B.(2010). Antidiarrheal activity of Dodonaea viscose root extracts, Int. J. Pharm. Bio Sci. 1 (4): 182-185.

Ruwart MJ, Kiepper MS, Bush BD (1980): Clonidine delays small intestinal transit in the rat. *J* Pharmacol Exp Ther 212: 487–490.

Teke G.N., Kuiate J. R. Ngouateu O. B. and Gatsing D. 2007. Antidiarrheal and antimicrobial activities of *Emilia Coccinea* (Sims) G. Don Extracts. J. Ethnopharmacol., 112: 278-283.

WHO / UNICEF Joint Statement (2004) - Clinical management of acute diarrhea. WHO / FCH / CAH / 04.7.

Niger. J. Physiol. Sci. 29(June 2014) 017 –022
www.njps.com.ng

Niger. J. Physiol. Sci.

Gender and environmental influences on visual acuity in Owerri, Nigeria

*** C.G. Emerole [1, 2], R. O. Nneli [2], E. E. Osim [2]**

[1]Optometry Unit, Eye Clinic, Federal Medical Centre, Owerri, Imo State, [2]Department of Physiology, College of Medicine, Abia State University, Uturu, Nigeria

Summary: This study assessed the gender and environmental influences on visual function among adults in Owerri, Nigeria. Visual acuity (V.A.) is a measure of visual function in health and disease. Visual disability together with other disabling conditions is a barrier to development, yet there is little known about the visual acuity and determinants of visual function in Owerri, Imo State, Nigeria. Results of a cross-sectional analytical study conducted between September 2007 and November 2009 using 3451 adults living in Owerri, Nigeria consisting of 2606 persons (test) and 845 persons(control), randomly selected are presented. Data were obtained using interviewer administered structured - questionnaires and standard procedures were used to determine gender and environmental influences on visual acuity. There were more females with poor vision than males in both study and control groups. The majority of the subjects were aged 40-49. At 6 metres, 20.9% and 39.1% of study and control groups in the right eye; 31.8% and 41.2% of study control groups respectively in the left eye had unaided V.A. ≥ 6/6. Similarly at 6m, 18.0% and 4.3% of study and control groups in the right eye; 15.2% and 5.0% of study and control groups respectively in the left eye had unaided V.A. < 6/18. Twenty-nine per cent and 25.0% of study and control groups respectively had unaided V.A. at near of N_5. Over 70.0% had ≤ N_6 at near and V.A. improvement with pin-hole device. Emmetropia was found in 20.5% (study) and 23.2% (control). The percentage prevalence of reduced VA was higher among ametropics and rural dwellers. The contributory factors were poor nutrition & irrational and uncontrolled use of chloroquine as first line drug for malaria treatment. Health education on diet, drug use and safe environmental health practices especially for persons living in rural areas in developing countries are recommended.

Keywords*:* Visual acuity, Vision, Gender, Age, Environment

**Address for correspondence:* coemerole@yahoo.com

Manuscript Accepted: April, 2014

INTRODUCTION

Vision is a vital physiologic parameter of ocular health. Traditionally, visual function is tested as visual acuity (capacity to discriminate fine details of objects and visual field (proportion of space in which objects are visible at the same moment during steady fixation of gaze in one direction). Colour vision, flicker sensitivity, contrast sensitivity, pupillary responses and motion testing are some of the other methods of quantitating vision (Attebo, 1996; Emerole et al, 2011; Johnson and Keller, 1983).Visual acuity test is a measure of central vision; a measure of sharpness of vision; and an assessment of total visual system from cornea to the occipital cortex. Visual acuity (V.A..) can be tested for both distant (far) and near vision. It is the most commonly used clinical measure for determining visual impairment (Borish, 1975). Visual acuity appears to reach its maximum at the age of 10, varies only slightly from the age of 15 to 20 onward until it begins to decline at the age of 45, as observed by most clinicians. However, more precise measures indicate that there is a gradual loss from that age onward, although the amount is not readily observed until an older age is reached, at which time morphological changes again influence it markedly, as they do in early years of life. In addition to changes in acuity, variations in retinal sensitivity with age also occur.Higher thresholds appear at the age of 40 and become even more pronounced by the age of 50 (Borish, 1975). Normal Vision (6/6) relies on the following: both eyes in alignment (extra-ocular muscles functioning); clear cornea; clear crystalline lens of the eye; clear ocular media (aqueous and vitreous); & intact retina, optic nerve and visual pathway. Saccadic eye movements are one of the factors that affect V.A.. (Borish, 1975; Emerole et al, 2011).Vision can be as low as counting fingers (CF); hand movement (HM); perception of light (PL) or even non-perception of light (NPL) (Borish, 1975; Emerole et al, 2013b).

Visual impairment is a result of abnormalities in the physiology of the eye or the visual pathway

(Guyton and Hall, 2003). This impairment can manifest in three predominant ways: a decrease in V.A.., a constriction of peripheral visual field, or an alteration in contrast sensitivity (Johnson and Keller, 1983). A visual acuity test is done as a diagnostic tool; to provide baseline data; as a measure of progression of disease; to evaluate treatment; as an employment and school admission requirements; in acquisition of license for motor driving and use of ammunition; for research purposes; and as a legal requirement (Emerole et al, 2013b; Emerole and Nneli, 2013). Amblyopia is defined as "reduced visual acuity not correctable by refractive means and not attributable to obvious structural or pathological ocular anomalies". Amblyopia can be primary (congenital), acquired (functional) and secondary (e.g toxic amblyopia). In persons malingering, there is a general inconsistency evident in the testing. The malingerer is generally uncooperative and ocular error does not correspond to the V.A. unlike in hysterical amblyopia (Borish, 1975). Factors such as age, refractive error, alcohol use, tobacco consumption, drugs, environmental factors (e.g dust, irritants, radiations, season), disease (systemic and ocular infections, allergies and disorders), illumination and nutritional deficiency may impair or affect vision (Anker, 1997, 1998; Borish, 1975; Carter et al, 2005; Emerole et al, 2011, Guyton and Hall, 2003; Lade, 2004; McClean et al, 1987; Miranda, 2011; World Bank, 2003).

Eye problems have been associated with self-reported visual function impairment and impairment in other activities of daily living. The first global estimate on the magnitude and causes of visual impairment was based on the 1990 world population data (38 million blind), indicating a two-fold increase in the magnitude of visual impairment in the world by 2020. This provided the basis for the 1999 launch of vision 2020, the global initiative for the elimination of avoidable blindness (World Health Organization, 1999). Visual disability, together with other disabling conditions, is a barrier to development (Bekibele et al, 2007; Fagbohungbe and Akinbode, 2012; Kirkwoood et al 1983; Nigeria Optometric Association Report, 2007; Nwosu and Alozie, 2006; The Eye Diseases Prevalence Group, 2004; World Health Organization, 1999; World Health Organization, 2000). Elimination of avoidable blindness is a moral imperative and priority under the disease control component of the Global Initiative – VISION2020: The Right to Sight among the poorest of the poor (World Health Organisation – News Report, 2000). This study was undertaken to provide epidemiologic information on gender and environmental influences on visual acuity among adults in Owerri, Imo state, Nigeria as a basis for cost effective intervention.

MATERIALS AND METHODS

Ethical approval for the study was obtained from Ethical committee of College of Medicine and Health Sciences, Abia State University, Uturu; and informed consent was obtained from the subjects. A total of 3451 subjects made up of 2606 persons of both sexes (test group) who met the inclusive criteria were recruited between September 2007 and November 2009 from a population of persons living in old Owerri province, Imo State, Nigeria. 845 persons both sexes (controls) were selected from a population of persons living in Ihiagwa autonomous community in Owerri–West local government area using a multistage random sampling. The controls were made up of individuals who did not present any eye defects on examination protocol. Only those above 18 years (aged 20–69 years) were selected for the study. Interviewer administered structured – questionnaires were used to determine demographic characteristics, dietary habits, alcohol consumption, tobacco use; and history of past ocular and systemic problems of subjects were also obtained. Those with conditions interfering with accurate ocular refraction such as corneal opacity; visually impairing opaque media; diabetes mellitus; hypertension; pseudophakic and aphakics were excluded from analysis.

All the subjects underwent a complete ophthalmic examination which included measurement of distant and near V.A.& pin-hole visual acuity in subjects with V.A. less than 6/6 (With Snellen's chart, near reading chart and tumbling ''E'' chart for illiterate subjects).Visual acuity at distant or far is reported as Snellen fraction (ratio between the distance at which the test is made or done in metres or feet [numerator] to the distance at which the smallest letter read subtends an angle of 50^0 [denominator] or with its decimal equivalent (e.g 6/12, 20/40, or 0.5). The Snellen fraction in meters is known as the metric snellen fraction and in feet as imperial fraction Vision in an eye were defined as above normal, >6/6; normal, 6/6; subnormal, <6/6 to 6/12; and visually impaired, ≤6/18.Reading fifty per cent or more of the letters on a line correctly was regarded as the subject getting the V.A. level correct. Improvement in visual acuity by two or more lines on the Snellen's chart when looking through the pin-hole was regarded as an indication of refractive error or uncorrected error when the the patient was already wearing spectacles);tonometry (tonometric values of 9mmHg – 24mmHg were taken as normal while tonometric values >24mmHg were considered clinically significant); ophthalmoscopy (internal eye examination was done with the direct ophthalmoscope); retinoscopy (refractive status was determined objectively with the streak retinoscope); subjective refraction; and perimetry to investigate other possible causes of impairment or reduction in

vision. Refractive errors (ametropia) in an eye were defined. Emmetropia was defined as a spherical dioptric power between -0.50DS and +0.50DS. Refraction data are based on subjective refraction.

Statistical analysis:
The data obtained were reported as percentages and statistical analysis was done using EPI Info version 3.5, 2008.

RESULTS

There were more females than males in both study and control groups. The majority of subjects were aged 40-49. There was no statistically significant difference between the control and the study group as shown in table 1.

In the present study, 61.2% and 61.3% of study and control groups respectively were domiciled in the rural areas. Forty-one per cent and 30.8% of study and control groups respectively had tertiary education. In the study group, students (22.9%) were in the majority while skilled persons (21.5%) were in the majority in the control group. About 57.4% of the subjects in the study group were unmarried while 49.7% of subjects in the control group were married (Emerole et al, 2011).

Twenty-one per cent and 39.1% of the subjects in the study and control groups respectively had unaided visual acuity at 6m ≥6/6 in the right eye (18.0% and 4.3% of study and control groups had unaided visual acuity of <6/18 in the right eye). There was no statistically significant difference in the unaided vision of the right and left eye visual acuity at 6m in the two groups as shown in table 2.

Table 1: Subjects by gender and age

Age (yrs)	MALE				FEMALE			
	A (n=1079)		B (n=357)		A (n = 1527)		B (n = 488)	
	No.	%	No.	%	No.	%	No.	%
20 – 29	194	18.0	32	9.0	340	22.3	89	18.2
30 – 39	183	17.0	50	14.0	260	17.0	122	25.0
40 – 49	279	25.8	170	47.6	441	28.9	189	38.7
50 – 59	244	22.6	65	18.2	296	19.4	47	9.6
60 - 69	179	16.6	40	11.2	190	12.4	41	8.4

A=Study group, B=Control group

Table 2: Unaided visual acuity at far (6m) of subjects

V.A. at 6m	**Oculus Dexter** (O.D/**Right eye**)				**Oculus Sinister** (O.S/**Left eye**)			
	A (n=2606)		B (n=845)		A (n=2606)		B (n=845)	
	No.	%	No.	%	No.	%	No.	%
>6/18	467	18.0	36	4.3	397	15.2	43	5.0
6/18	454	17.4	56	6.6	384	14.7	49	5.8
6/12	725	27.8	197	23.3	652	25.0	164	19.4
6/9	414	15.9	226	26.7	344	13.2	242	28.6
6/6	290	11.1	183	21.7	482	18.5	176	21.0
6/5	256	9.8	147	17.4	347	13.3	171	20.2

A=Study group, B=Control group, V.A.= Visual Acuity

Majority (70.9% and 75.0% of study and control groups respectively) had unaided near visual acuity less than N_5. The differences between the study and control groups were not statistically significant as shown in table 3. The unaided vision in 79.5% and 76.5% of study and control groups was improved with pin-hole. The differences between the study and control groups were not statistically significant as shown in table 4. The difference in gender and age between the study and control groups with respect to emmetropia was not statistically significant. Only 20.5% and 23.2% of the study and control groups respectively were emmetropic. The prevalence of emmetropia decreased with age as shown in table 5.

Ametropia and risk factors affecting visual acuity

Majority of the subjects (79.5% and 76.6% of the study and control groups respectively) had ametropia In the present study, 67.6% and 49.2% of ametropics in the test and control groups respectively used quinines as first-line drug for malaria while 62.2% and 31.0% of study and control groups respectively were in the habit of consuming food items of low nutritional value. About 33.6% and 27.4% ametropics in the study and control groups respectively consumed alcohol while 19.9% and 15.1% of ametropics in the study and control groups respectively had past history of ocular trauma (Emerole et al, 2011).

Table 3: Unaided visual acuity at near (40cm) – oculus uniter/ou/both eyes of subjects

V.A. at 40cm	A (n = 2606)		**B** (n = 845)	
	No.	%	No.	%
N_5	758	29.1	211	25.0
N_6 - N_{18}	1227	47.1	441	52.2
<N_{18}	621	23.8	193	22.8

A=Study group, B=Control group, V.A.= Visual Acuity

Table 4: Pin-hole visual acuity (visual improvement with pin-hole in the unaided eye of subjects)

Pin-hole V.A. Improvement	**A** (n = 2606)		**B** (n = 845)	
	No.	%	No.	%
YES	2072	79.5	649	76.8
NO	534	20.5	196	23.2

A=Study group, B=Control group, V.A.= Visual Acuity

Table 5: Emmetropia By Gender And Age

Age (yrs)	**MALE**				**FEMALE**			
	A (n=252)		B (n= 91)		A (n = 282)		B (n = 105)	
	No.	%	No.	%	No.	%	No.	%
20 – 29	46	18.3	8	8.8	55	19.5	17	16.2
30 – 39	42	16.6	12	13.2	51	18.1	29	27.6
40 – 49	62	24.6	43	47.2	71	25.2	36	34.3
50 – 59	56	22.2	15	16.5	65	23.0	13	12.4
60 - 69	46	18.3	13	14.3	40	14.2	10	9.4

A=Study group, B=Control group

DISCUSSION

The socially constructed nature of gender and culturally based expectations of the roles and behaviours of women and men mediates health inequalities, and the old adage "women get sicker but men die quicker" (Anker, 1997, 1998). Many tasks associated with rural living are divided along gender lines. Gender roles unlike the biology of sex are dynamic-(can change over time and vary widely within and across a culture). Some aspects of these roles originated in the biological differences between the sexes (Anker, 1997, 1998; Miranda, 2011; World Bank, 2003). There were more females than males in this study (58.6% and 57.8% of the study and control groups respectively). This agrees with Nworah and Ezepue, (1992); Nwosu and Alozie, (2006); & Patel and West, (2007). The complaint of reduced vision was more among the females and rural dwellers in this study. Gender differences and environmental influences were observed in the prevalence of ametropia and visual development (Borish, 1975; Emerole et al, 2011). In the rural settings, women may be more exposed to ocular hazard and pollution of the environment from use of firewood for cooking, bush burning practices, cow's skin and hides, long exposure to ultra-violet rays from sunlight during farming, use of lantern or candle to read and dusty environment especially during harmattan and dry season (Emerole et al, 2011). The apparent higher visual demand on women from gender roles may explain why more females in this study sought intervention.

Age is a physiologic parameter and of medical significance in diagnosis and in determining treatment. In this study, the majority of the subjects were aged 40-49 years. In a young person, the crystalline lens is flexible allowing the eye to focus on objects at far and increases its focusing power (accommodation) if objects are brought close. There is a gradual decline in the flexibility of the crystalline lens and accommodation after the age of 35. This presents with varying degrees of difficulty in clear, effortless and comfortable vision at far and near (Borish, 1975). In the present study, the most dorminant ametropia was hyperopia (35.2% and 34.7% in the study and control group with no significant difference between the two groups, $p = 1.00$). The decline in vision at near from presbyopia and hyperopia as the lens becomes rigid (making seeing at close range more difficult) may explain why majority of those who presented for visual care due to reduced vision were in this age group. Ageing also modifies a pre-existing error making it more symptomatic (Borish, 1975; Emerole et al, 2011; Nworah and Ezepue, 1992).

Visual function is affected by gender; age; ocular refraction; alcohol and tobacco consumption; drugs; systemic and ocular diseases, disorders and allergy; environmental factors (such as illumination, dust, irritants and radiation); and nutritional deficiencies (Borish, 1975; Carter et al, 2005; Emerole et al, 2011). In the present study, 20.9% and 39.1% of study and control groups respectively had unaided V.A. at 6m of $\geq$ 6/6 in the right eye while 31.8% and 41.2% of study and control groups respectively had unaided V.A. at 6m of $\geq$ 6/6 in the left eye. Similarly, 18.0% and 4.3% of study and control groups respectively had unaided V.A. at 6m of <6/18 in the right eye while 15.2% and 5.0% of study and control groups respectively had unaided V.A. at 6m of <6/18 in the left eye. In the study on prevalence of blindness and visual impairment in Atakunmosa local government area of south western Nigeria; South Sudan, and school children in Tanzania 6.30%, 7.70% and 9.50% respectively had presenting vision <6/18 in their better eye/eye with better vision or V.A. (Kingo and Ndawi, 2009; Ngondi et al, 2006; Onakpoya et al, 2007). Majority of the subjects (over 70.0% of the study and control groups) had V.A. improvement with pin-hole device/aid in the unaided eye. This is indicative of presence of uncorrected refractive error (Borish, 1975).The magnitude of pin-hole visual acuity improvement is consistent with the prevalence of ametropia in this study.Twenty-nine per-cent and 25.0% of study and control groups respectively had unaided V.A. at near of N_5 (over seventy per cent of subjects in the study and control groups had unaided V.A. of $\leq N_6$ at near). This is consistent with the magnitude of presbyopia in the study and control groups. The observations in this study are consistent with earlier studies on prevalence and impact of presbyopia in low and middle income countries (Emerole et al, 2011; Kingo and Ndawi, 2009; Ngondi et al, 2006; Onakpoya et al, 2007). The findings suggest that more than half of adults over the age of 30 have presbyopia; women have both a higher prevalence of, and more severe presbyopia; hotter climates are associated with the earlier onset of presbyopia and the majority of those with presbyopia (an aspect of refractive error that impacts on older persons economic independence) do not have corrective spectacles. This observation corroborates the findings of Bekibele et al, (2007) & Patel and West, (2007).

The matter of the dominant eye is usually of importance from a diagnostic consideration. While opinion varies from those who believe that ocular and hand dominancy is of little significance to those who ascribe to it, most of the defects of personality and asthenopia. Many investigators seem to indicate that the dominant eye has a definite place in the determination of proper refractive correction. It is believed that opposite handedness and eye dominancy is less efficient than coincident dominancy (Rengstorff, 1967). Fagbohungbe and Akinbode (2012) recommend that

those who engage in any visual acuity search task that requires monocular viewing should always identify and use their dominant eye for effective performance. In a study on dominance, Rengstorff (1967) found 91.5% right-handed, 7.7% left-handed, 1.1% ambidextrous, 66.0% right-eyed and 34.0% left-eyed. Also in a separate study of mentally retarded children, Rengstorff reported that 51.0% of subjects indicated mixed hand and eye relationship (Borish, 1975; Rengstorff, 1967).From above observations, majority of individuals are right handed, thus right handedness associated with left eyedness as a result of uncorrected refractive error and reduced vision implies alteration of the dominancy of an eye from the natural one to the other. This will result in difficulties of personality and comfort and may explain why subjects in the study group sought intervention. There were also more subjects in the study group with unaided V.A. at 6m of <6/18 than in the control group.

In the present study, majority of the (79.5% and 76.8% of study and control groups respectively) had ametropia/refractive error. A refractive error is an error in the focusing of light by the eye and a frequent reason for reduced visual acuity; and a major cause of visual impairment and blindness. Of the 2606 subjects in the study group and 845 subjects in the control group in this study, 35.2% and 34.7% of study and control groups had hyperopia; 23.4% and 19.6% of study and control groups were myopic; 20.9% and 22.5% of study and control groups had astigmatism. Ocular refractive status is considered normal ("emmetropia"), if parallel light rays from distant objects are in sharp focus on the retina when the ciliary muscle is completely relaxed. Emmetropia was found in 20.5% and 23.2% of the study and control groups respectively. Although there were more emmetropic subjects in the control group than study group, it was not statistically significant and decreased with age. Emmetropia of 50.6% was found in the Chennai glaucoma study in India. Prevalence of emmetropia was higher in studies in the United States of America and Asia (Emerole et al, 2011; Raju et al, 2004; The Eye Disease Prevalence Research Group, 2004; World Health Organization, 1999; World Health Organization, 2000).Studies conducted in the United States of America, Western Europe and Australia showed prevalence of ametropia of 35.1%, 37.7% and 22.2% respectively. This appears paradoxical as demographic studies have shown that Asia and America harbour more aged population and thus are expected to have a higher prevalence of ametropia since increase in age is a physiologic determinant of ametropia. This observation shows that the environment may be a factor in this high prevalence of ametropia and visual acuity pattern (Raju et al, 2004; The Eye Diseases Prevalence Research Group, 2004).

The use of chloroquine as anti-malarial as well as poor dietary habits among the subjects with reduced vision in the study and control groups, were statistically significant. The eyes need adequate nutrition to function as other parts of the body (Emerole et al, 2011; Emerole et al, 2013a; Lade, 2004). Drugs are casually referred to as 'useful poisons'. About sixty-seven per cent and forty-nine per cent of ametropics and subjects that had reduced vision in the study and control groups respectively used chloroquine as their regular anti-malarial. Malaria is endemic in Nigeria and Chloroquine is used as first line drug especially in the rural areas. Abuse of chloroquine can damage the macula and affects components of refraction especially in the absence of adequate nutrition (Borish, 1975). Many of the ametropics (62.2% in the study group and 31.6% in the control group) were in the habit of consuming food items of low nutritional values. From this study, poor dietary habit and abuse of chloroquine may be implicated as risk factors for reduced V.A. (Emerole et al, 2011).

This study has provided information on gender and environmental influences on vision among adults in Owerri, Imo state, Nigeria. The V.A. pattern is consistent with the magnitude of ametropia and presbyopia in the study and control groups. The differences in visual acuity when compared with high and medium resource countries may be due to the high prevalence of ametropia, identified poor level of nutrition, frequent use of chloroquine derivatives for treatment of malaria as well as being domicile in the rural areas. Regular vision screening and health education on diet, drug use and safe environmental health practices are recommended. In conclusion ametropia was highly endemic in the population owing to poor nutrition, regular use of chloroquine and environmental factors; and that more females than males presented with higher rate and more severe eye defects like presbyopia.

REFERENCES

Anker R (1997): Theories of occupational Segregation by sex: an overview. International Labour Review, 136: 315-339.

Anker R (1998): Gender and jobs: Sex segregation of occupations in the world, Geneva: International Labour Organization (ILO), Cambridge Publication, paper, xi + 456pp.

Attebo K, Mitchell P, Smith W (1996): Visual acuity and the causes of visual loss in Australia: The Blue Mountain's Eye study. *Ophthalmology*; 103: 357 – 364.

Bekibele C.O, Fawole O.I, Bamgboye A.E, Adekunle L.V, Ajayi R, Baiyeroju A.M (2007): Prevalence of refractive errors and Attitude to spectacle use among drivers of public institutions in Ibadan, Nigeria. *Annals of African Med.* Vol. 6, No. 1, pp.26 – 30.

Borish I.M (1975): Clinical Refraction. The Professional Press, Inc. Chicago, Illinois. 3rd edition.Pp. 5 – 694.

Carter C.R, Jacobson S.W, Molteno C.D, Chodo L.M, Vljo D, Jacobson J.L (2005): Effects of Prenatal alcohol exposure on infant visual acuity. *J Pediatr.*147: 473 –9.

Emerole C.G, Nneli R.O, Osim E.E, Anyaehie U.B, Okereke C (2011): Prevalence, Determinants and

Distribution of Refractive Errors in a Nigerian Community. *J. Biosciences Biotechnology Research Asia.* Vol. 8(2), p. 523 – 528.

Emerole C.O, Emerole C.G, Chukwuocha U.M (2013a): Nutritional intervention in HIV/AIDS management – An overview. *American Health Advocacy Publishing, Health Policy*; 14: 5 – 12.

Emerole C.G, Emerole C.O, Nneli R.O (2013b): Blindness and low vision in Nigeria: A Review of Etiological factors and Impact. *J.Hlth. & Vis. Sci.* Vol. 15(1): 10-15.

Emerole C. G, Nneli R.O, (2013): Visual Indices of motor vehicle drivers in relation to road safety in Nigeria. *Niger. J. Physiol. Sci.* 28: 057 – 062.

Fagbohungbe, B.O, Akinbode G.A (2012): Task difficulties, eye conditions and visual acuity tasks among selected students of the Universityof Lagos, Nigeria. *British Journal of Arts and Social Sciences.* ISSN: 2046 – 9578, vol. 6 no. 1.

Guyton A.C, Hall J.E (2003): Text book of Medical Physiology 10th ed. Elsevir, New Delhi, India pp. 566 – 800.

Johnson C.A, Keller J.L (1983): Incidence of visual field loss in 20, 000 eyes and its relationship to driving performance. *Arch ophthalmology*. 101: 371 – 375.

Kingo A.U, Ndawi B.T (2009): Prevalence and causes of low vision among school children in KibahaDistrict Tanzania. *Journal of Health Research* ISSN: 1821 – 6404. Vol. 11. No. 3, pp 111 – 115.

Kirkwood B, Smith P, and Marshall T, Prost T (1983): Relationships between mortality, visual acuity and micro filarial load in the Onchoceraiasis Control Program. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 77, 862 – 868.

Lade A.A (2004): Effects of Smoking/Nicotine on Night Vision.http://www.aalade.com /resource/OBE01htm. 11: 3 – 8. Retrieved 14/02/13.

McClean S, Parsons R.S, Chesterman R.B, Dinear R, Johnson M.G, Davies N.W (1987): Drugs, Alcohol and Road accidents in Tasmania. *Med. J.* 147: 6 – 11.

Miranda V (2011): Cooking, Caring and Volunteering: Unpaid work around the world, Organization -for Economic Co-operation and Development (OECD) Social, Employment and Migrations Working papers, No. 116, OECD Publishing. dot:10.171/5Kghrjm8s142–en.Retrieved 03/03/2011.

Ngondi J, Ole – Sempele F, Onsarigo A, Matende I, Baba S, Reacher M et al (2006): Prevalence and causes of blindness and low vision in Southern Sudan. *PLOS Med.*, 3: e477.

Nigerian Optometric Association (2007): NOA report on the strategic action plan for VISION 2020: The right to sight for Nigeria, 2007 – 2011.

Nworah P.B, Ezepue U.F (1992): Prevalence of errors of refraction in a Nigerian eye clinic. *Orient J. of Med.*; 4: 57 – 60.

Nwosu S. N. N, Alozie I.U (2006): Refractive Errors in school children in Onitsha, Nigeria. *Nig. J. of Hlth. & Biomed.Sci.*vol. 5. No. 1, pp. 114 – 117.

Onakpoya O.H, Adeoye A.O, Akinsola F.B, Adegbehingbe B.O (2007): Prevalence of blindness and visual impairment in Atakunmosa West local Government Area of South Western Nigeria. *Tanzania Health Research Bulletin.* ISSN: 0856 – 6496. Vol. 9, No. 2, pp. 126 – 131.

Patel I, West S.K (2007): Impact of presbyopia on quality of life in a rural African setting. *Ophthalmol. 113 (5): 728 – 734.7.*

Raju P, VeRamesh S, Arvind H, George R, Baskaran M, Paul P.G, Kumaramanickavel G, McCarty C,Vijayal (2004): Prevalence of Refractive Errors in a Rural South Indian Population. *Inv. Ophthalmol. & Vis. Sci.*, vol. 45, No., 12: 4268 – 4271.

Rengstorff R.H (1967): Variations in myopia measurement: An after – effect observed with habitual wearers of contact lenses. Arch. Am. Acad. Optom., 44:149.

The Eye diseases Prevalence Research Group (2004): Prevalence of refractive errors among adults in the United States, Western Europe and Australia. *Arch Ophthalmol.* 122: 495 – 505.

World Bank (2003): Gender Equality and the Millenium Development Goals. Gender and Development Group, Washington: World Bank.04 April 2003, p. 3 – 6.

World Health Organisation (1999): VISION 2020: THE RIGHT TO SIGHT. Director – General launches a global initiative to combat avoidable blindness. Press releaseWHO/12.Available from:http://www.vision2020.org/documents/WHO

World Health Organisation (2000): Elimination of avoidable visual disability due to refractive error. WHO/PBL/00.79, 1 – 54. *Report Geneva: WHO.*

Niger. J. Physiol. Sci. 29(June 2014) 029 –036
www.njps.com.ng

Niger. J. Physiol. Sci.

Hepatoprotective and anticlastogenic effects of ethanol extract of *Irvingia gabonensis (IG)* leaves in sodium arsenite-induced toxicity in male Wistar rats

*** Gbadegesin, M. A., Adegoke, A. M., Ewere, E. G. and Odunola, O. A.**
Cancer Research and Molecular Biology Unit, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria

Summary: Consumption of arsenic contaminated water has been associated with diverse health defects such as cancer and skin lesions. Some plants of medicinal value have been reported to show protective effects against toxins. In this study, the effects of ethanol extract of the leaves of *Irvingia gabonensis* (IG) against sodium arsenite (SA) induced hepatotoxicity and clastogenicity in male Wistar rats was investigated. Eight groups of five rats each were used for the study. They were administered with 250 or 500 mg/kg body weight of IG with or without SA at 2.5 mg/kg body weight. IG extract has a significant ($p<0.05$) reducing effect on serum liver function enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (γGT) activities. This was corroborated with the histopathological analysis findings. Also the groups treated with both the extract and SA recorded significantly ($p<0.05$) reduced number of micronuclei when compared with the group treated with SA only. IG extract also reduced the oxidative stress induced by SA as measured by the reduced generation of hydrogen peroxide (H_2O_2) and significant ($p<0.05$) difference in the CAT and SOD activities between the groups treated with both SA and extract, and the positive control group administered SA alone. This study therefore shows that the ethanol leaf extract of *Irvingia gabonensis* have hepatoprotective and anticlastogenic effects against sodium arsenite-induced toxicity possibly by enhancing the antioxidant status in the Wistar rats.

Keywords: *Irvingia gabonensis* , Sodium arsenite, Hepatotoxicity, Clastogenicity, Oxidative stress, transaminases.

**Address for correspondence:* magbadegesin@yahoo.com Tel.: +234 7031975907

Manuscript Accepted: March 2014

INTRODUCTION

Cancer causing substances damage DNA and induce transmittable mutations in both humans and animals (Perera *et al*., 2002). Their interaction with tissue macromolecules such as DNA alters the replication and repair mechanisms (Ames, 1979). For instance, sodium arsenite, a common contaminant of underground water in some regions of the world, is known to be hepatotoxic and genotoxic (Guillamet *et al.*, 2004; Odunola *et al.*, 2008; Gbadegesin *et al.*, 2009).

Attentions have recently shifted to the use of medicinal plants in prophylactic, therapeutic and curative applications. *Irvingia gabonensis* (IG) (AubryLecomte ex O'RorkeBaill) is a plant with promise. It is an indigenous forest tree belonging to the group of plants classified as non-timber forest products. *Irvingia gabonensis* is commonly called bush mango or African mango since the trees bear fruits that look like small mango (Matos *et al*., 2009). Traditionally, the stem bark is used to relieve pain in Sierra Leone (Okolo *et al*., 1995). The aqueous maceration of the leaves is used as antidote for some poisonous substances. In combination with palm oil, the leaves are also used to stop haemorrhage in pregnant women. In Senegal, the decoction of the stem bark is used in the treatment of gonorrhoea, hepatic and gastrointestinal disorders (Hubert *et al*, 2010). The root bark is prescribed in poultrice form to treat wounds. The decoction of the root bark is also used to treat diarrhoea (Osadebe *et al*., 2012). The nut is used in treating type 2 diabetes (Adamson *et al.*,1990). The kernel serves as condiments used in thickening and flavouring soups. The fruit is consumed in the south-western part of Nigeria with less interest in the nuts while the reverse is the case in the eastern part. The stem bark of the tree is added to palm wine as a preservative (NAERLS, 1999). The leaf extracts of the plant has been reported to have

diuretic effect in rats and hypotensive effect in cats (Nosiri *et al.,* 2009a; 2009b)

However, there is dearth of information on the hepatoprotective, antioxidant and anticlastogenic effects of the leaves of this plant. This study was therefore conducted to evaluate the hepatoprotective, antioxidant and anticlastogenic effects of the ethanol extract of *Irvingia gabonensis* (AubryLecomte ex O'RorkeBaill) leaves on sodium arsenite-induced toxicity in male Wistar albino rats.

MATERIALS AND METHODS

Reagents and kits

Sodium arsenite ($NaAsO_2$; BDH chemicals Ltd poole England) was dissolved in distilled water and administered at a dose of 2.5 mg/kg body weight corresponding to $1/10^{th}$ of the oral LD_{50} of the salt (Preston *et al.,* 1987). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (γGT) kits were obtained from Randox Laboratories, Crumlin, UK. All other chemicals and reagents were of analytical grade and were products of Sigma Chemical Co. St. Louis, MO., USA or BDH Chemical Ltd, Poole, England.

Test plant material and extraction procedure

Fresh leaves of the plant were harvested, identified and Voucher specimen deposited at the herbarium of the Department of Botany, University of Ibadan. The harvested leaves were air-dried in a well-ventilated room for about four weeks, before milling. Cold extraction was then carried out on the grounded sample by soaking in 50% ethanol for 72 hours at room temperature. The extract was filtered and the filtrate concentrated using a rotary evaporator at temperature of 40 ^{0}C. The concentrated sample was then freeze-dried and the sample kept at room temperature and administered to the experimental animals as detailed below.

Experimental animals and treatments

Forty male Wistar albino rats weighing between 150 – 190 g raised at the preclinical experimental animal house, Department of Physiology, were purchased and kept in the experimental animal facility of the Department of Biochemistry, University of Ibadan. They were fed with commercial rat pellets (Ladokun Feeds, Mokola, Ibadan, Nigeria) and water *ad libitum*.

The albino rats were allowed to acclimatize for one week prior to the commencement of the study. The rats were randomly distributed into eight groups of five animals each based on the treatment received.

Group 1: These served as control and were administered distilled water only.

Group 2: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite for 2 weeks.

Group 3: The rats in this group were orally intubated daily with 250mg/kg body weight of *Irvingia gabonensis* (IG) extract for 2 weeks.

Group 4: The rats in this group were orally intubated daily with 500mg/kg body weight IG extract for 2 weeks.

Group 5: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite and 250mg/kg body weight IG extract simultaneously for 2 weeks

Group 6: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite and 500mg/kg body weight plant extract simultaneously for 2 weeks.

Group 7: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite for 2 weeks followed by 250mg/kg body weight IG extract for subsequent 2 weeks.

Group 8: The rats in this group were orally intubated daily with 2.5mg/kg body weight sodium arsenite for 2 weeks followed by 500mg/kg body weight IG extract for another 2 weeks.

Termination of the experiment and extraction of tissues

The rats were sacrificed by cervical dislocation twenty four hours after the last treatment dose. 0.04% colchicine was administered at a dose of 10 ml/kg body weight to the animals 2 hours prior to the sacrifice. Blood samples were collected through retro-orbital bleeding, and put into a plain bottle and allowed to clot. The samples were then centrifuged at 3,000 g for 30 minutes to separate the serum.

The animals were dissected and opened to harvest the liver and femur. A portion of the liver from each sacrificed animal was excised, blotted and then perfused with potassium chloride (1.15%) in order to remove all traces of blood haemoglobin which might contaminate the tissues. The liver samples were preserved and fixed in 10 % buffered formal-saline and processed for paraffin sectioning. Sections of about 5 µm thickness were stained using Haematoxylin-Eosin staining dye and subsequently evaluated in the Veterinary Pathology Department of the University of Ibadan. The remaining portion of each liver sample was homogenized in phosphate buffer and centrifuged at 4 ^{o}C. The supernatant was used immediately or stored at – 20 ^{o}C. The femurs were also excised and bone marrow extruded for micronucleus induction assay.

Assays of liver function enzymes

γ-glutamyltransferase activity.

γGT was assayed in the serum by using the reconstituted γGT diagnostic reagent following the method of (Szasz, 1969). The principles involve the transfer of glutamyl group from a glutamyl peptide (L-γ-glutamyl-p-nitroanilide) to another peptide (glycylglycine), in a reaction catalysed by γGT, thereby yielding a cleavage product (pnitroaniline).

This product absorbs UV at 405 nm thus making a direct kinetic determination of γGT activity possible.

Alanine aminotransferase and aspartate aminotransferase activities

Serum ALT and AST were assayed according to (Reitman and Frankel, 1957) using commercial diagnostic kits. This method involves the reaction of pyruvate, the product of transamination reaction catalysed by ALT or AST, with 2, 4 -dinitrophenyl hydrazine to produce intensely coloured hydrazone read at 546 nm using a spectrophotometer (Spectronic-20).

Histological Analysis

Liver sections were fixed in 4% p-formaldehyde and washed in phosphate buffer pH 7.4 at 4^0C for 12 hours. After dehydration, the tissue was embedded in paraffin, cut into sections, stained with haematoxylin–eosin dye and finally observed under a microscope.

Micronucleus (MN) assay

The femurs from each of the animals were removed and bone marrow was aspirated. Microscopic slides of the bone marrows were prepared according to (Matter and Schmid, 1971). The slides were then fixed in methanol, air-dried, pre-treated with May-Grunwald solution and air-dried again. The dried slides were stained in 5 % Giemsa solution and induced in phosphate buffer for 30 seconds. Thereafter, it was rinsed in distilled water and air-dried. The slides were mounted and scored under a microscope for micronucleated polychromatic erythrocytes (mPCEs).

Antioxidant enzymes and hydrogen peroxide generation assays

H_2O_2 generation assay

50 µl of supernatant fraction of the liver homogenate was vortexed and incubated at room temperature for 30 minutes. This was then shaken to ensure proper mixing and incubated for 30minutes before reading at 560 nm. The concentration of the hydrogen peroxide generated was extrapolated from the standard curve.

Determination of catalase activity

1 ml of supernatant fraction of the liver homogenate was mixed with 19 ml distilled water to give a 1: 20 dilution. The assay mixture contained 4 ml of H_2O_2 solution (800 µmoles) and 5 ml of phosphate buffer, pH 7.0 in a 10 ml flat bottom flask. 1 ml of properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1 ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide contents of the withdrawn sample were determined by the method described above.

Determination of Superoxide dismutase (SOD) activity.

1 ml of supernatant fraction of the liver homogenate was diluted in 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Statistical analysis:

The results were expressed as mean ± Standard deviation. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 10.0.1. P-values less than 0.05 were considered statistically significant for differences in mean.

RESULTS

Effect of *Irvingia gabonensis* on the body and liver weights of rats

The initial body weights of the animals were taken before the commencement of treatments. Just before sacrifice, the body weights of the animals were taken and designated as final body weights. These were used to calculate the percentage body weight change. The harvested liver tissues were also weighed and the relative liver weights were determined (Table 1).

Administration of sodium arsenite caused a significant ($p<0.05$) reduction in the percentage weight change as compared with the negative control. There were no significant ($p>0.05$) change in the body weight in other groups, after the four weeks of treatment, as compared with the negative control (Table 1). Also, changes in the relative liver weight of rats were not significant ($p> 0.05$) when compared across all groups.

Hepatoprotective activities of ethanol extract of the leaf of *Irvingia gabonensis* in Wistar rats treated with sodium arsenite.

The protective effect of *Irvingia gabonensis* leaf extract was assessed by evaluating the activities of serum enzymes; γ-glutamyltransferase (γGT), aspartate amino transferase (AST) and alanine amino transferase (ALT) in the groups of rats administered the extract and/or sodium arsenite and the control untreated rats. Administration of sodium arsenite at 2.5 mg/kg body weight resulted in almost double folds increase in the mean serum AST value, more than double ALT value and more than fourfold

increase in γGT when compared with negative control group.

The mean ALT, AST and γGT activities in groups of rats administered graded doses of *Irvingia gabonensis* extract with sodium arsenite decreased significantly ($p<0.05$) when compared with the group treated with sodium arsenite alone (Table 2). The extract alone at the high dose of 500 mg/kg body weight produced significant ($p<0.05$) higher level of serum AST and ALT activities. The results of the histological assessment of the liver cells integrity support the serum enzyme activities patterns of hepatotoxicity in the treated animals (Figure 1). There was marked widespread thinning of hepatic cords in the liver of rats administered sodium arsenite (positive control). There were no visible lesion in the negative control group and the groups administered 250mg/kg and 500mg/kg body weights of the extract (groups 1, 3 and 4 respectively). There were no observable lesions in the groups 5 and 6, administered 250 and 500 mg/kg of extracts respectively with sodium arsenite at 2.5mg/kg. However, there is multifocal centrilobular thinning of hepatic cords in group 7, and moderate Kupffer cell hyperplasia, portal fibrosis and random single-cell necrosis in group 8. Groups 7 and 8 were post-treated with extracts at 250 and 500 mg/kg respectively, after two weeks of sodium arsenite administration.

Effect of *Irvingia gabonensis* on the frequency of micronucleated polychromatic erythrocytes (mPCEs) scored in the bone marrow of rats treated with sodium arsenite.

The number of mPCEs per 1000 PCEs scored in the

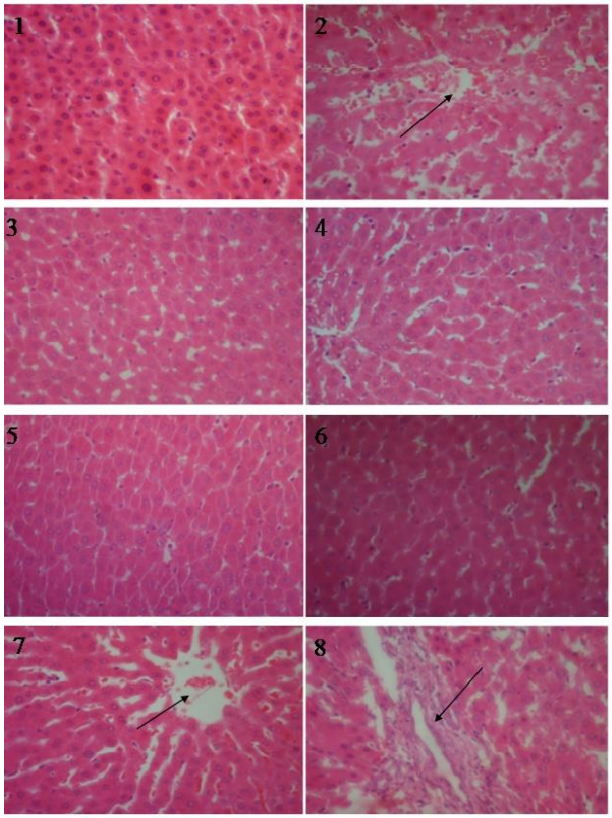

Figure 1. Representative photomicrograph of liver of rats treated with sodium arsenite and/or *Irvingia gabonensis* extract (Magnification= x400). Group numbers are shown. **1** (group given distilled water only). There are no visible lesion. In group **2**, given sodium arsenite, there is marked widespread thinning of hepatic cords. There are no visible lesions in groups 3, 4 (given extract, respectively at 250 and 500mg/kg body weight), and groups 5 and 6 treated with sodium arsenite along with the extract. There is multifocal centrilobular thinning of hepatic cords in group 7. There is moderate Kupffer cell hyperplasia; portal fibrosis and random single-cell necrosis in group 8. Groups 7 and 8 were post treated with the extract at 250 and 500mg/kg body weight respectively after treatment with sodium arsenite for 2 weeks.

Table 1. Body weights, liver weights and percentage body weight changes of experimental rats (values are mean ±SD).

Group/ Treatment	Initial **B.Wt** (g)	Final B.Wt (g)	%Δ**Wt** (g)	Liver W**t**t(g)	**Relative** Liver W**t** (%)
1. Dist. water	156.00±26.07	206.00±26.07	28.66±7.14	5.29±1.00	2.56±0.25
2. SA	188.00±10.95	208.00±4.47	14.82±3.21#	5.76±0.61	2.77±0.25
3.250mg/kg IG extract	156.00±5.48	194.00±13.42	27.39±6.07	5.04±0.71	2.59±0.25
4.500mg/kg IG extract	160.00±0.00	202.00±17.89	29.69±9.37	5.52±0.84	2.72±0.22
5.SA+250mg/kg IG extract	164.00±16.73	198.00±10.95	23.81±5.05	5.15±0.32	2.60±0.13
6.SA+500mg/kg IG extract	160.00±0.00	186.00±19.49	20.31±9.37	4.88±0.84	2.62±0.47
7.SA(2weeks)+ 250mg/kg extract	156.67±11.55	178.00±10.00	22.51±4.75	5.51±0.31	2.89±0.01
8.SA(2weeks)+ 500mg/kg extract	116.00±8.94	170.00±26.46	27.78±9.62	5.09±0.86	2.64±0.31

SA = Sodium Arsenite, IG = *Irvingia gabonensis* # = The mean difference is significant ($p< 0.05$) when compared with control

Table 2. Levels of serum gamma-glutamyltransferase, aspartate aminotransferase, and alanine amino transferase in experimental rats treated with SA and/or *Irvingia gabonensis* extract (values are mean± S.D).

Group/Treatment	AST(U/l)	ALT(U/l)	γGT(U/l)
1. Dist. water	41.84±1.89	8.75±2.13	2.32±1.15
2. SA	78.61±11.84#	22.99±3.36#	10.42±1.15#
3. 250mg/kg IG extract	51.44±18.69	16.78±10.98	3.09±1.76
4. 500mg/kg IG extract	72.00±19.10#	12.79±1.62#	11.19±9.98
5. SA+250mg/kg IG extract	52.61±8.13*	9.00±5.80*	5.21±4.38*
6. SA+500mg/kg IG extract	57.24±12.49*	9.46±6.17*	3.47±3.27*
7. SA (2weeks) + 250mg/kg extract	40.72±9.36*	9.23±1.65*	6.94±3.47
8. SA (2weeks)+ 500mg/kg extract	39.65±6.64*	8.92±2.69*	4.63±1.83*

SA = Sodium Arsenite, IG = *Irvingia gabonensis.* # = The mean difference is significant ($p< 0.05$) when compared with control (group 1). *= The mean difference is significant ($p< 0.05$) when compared with group treated with SA alone (group 2).

Table 3. Intracellular levels of hydrogen peroxide generated, catalase and superoxide dismutase activities in the liver of rats treated with sodium arsenite and/or *Irvingia gabonensis* extract (values are mean ± SD).

Treatment	H_2O_2 (µmol/min./mg protein)	CAT (Units/mg protein)	SOD (Units/mg protein×10^{-2})
1. Dist. water	13.81±1.14	65.21±3.40	0.14±0.02
2. SA	17.29±2.89#	76.53±4.29#	0.13±0.004
3.250mg/kg IG extract	13.29±0.64	67.66±7.99	0.15±0.05
4.500mg/kg IG extract	14.54±0.27	65.90±11.18	0.15±0.03
5.SA+250mg/kg IG extract	14.09±1.14*	71.31±7.46	0.15±0.02
6.SA+500mg/kg IG extract	15.06±0.66	70.11±5.09	0.15±0.01*
7.SA(2weeks)+ 250mg/kg extract	16.81±2.91	65.92±6.06*	0.15±0.03*
8.SA(2weeks)+ 500mg/kg extract	15.00±1.79	67.51±6.94*	0.15±0.02*

SA = Sodium Arsenite, IG = *Irvingia gabonensis.* # = The mean difference is significant (p< 0.05) when compared with control (group 1). *= The mean difference is significant (p< 0.05) when compared with group treated with SA alone (group 2).

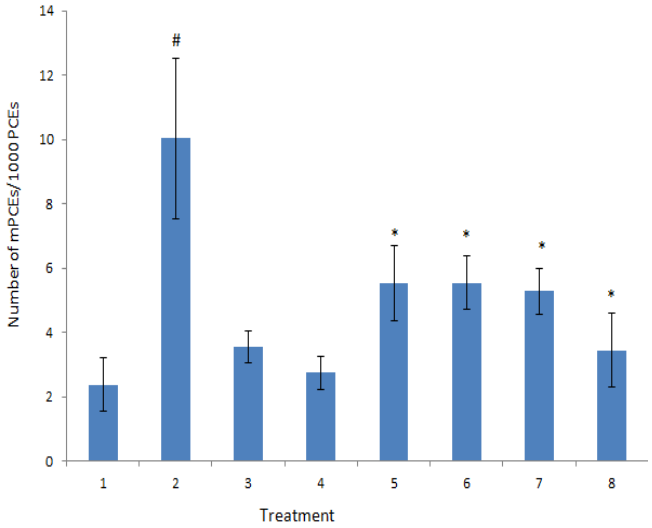

Figure 2. Frequency of micronucleated polychromatic erythrocytes (mPCEs) scored in the bone marrow cells of rats treated with sodium arsenite and/or *Irvingia gabonensis* extract (IG). Group 1 Negative control given distilled water only. Group 2: Given only sodium arsenite (at 2.5mg/kg body weight), Group 3: 250mg/kg body weight (IG). Group 4: 500mg/kg body weight IG, Group 5: Sodium arsenite and 250mg/kg body weight IG simultaneously, Group 6: Sodium arsenite and 500mg/kg body weight IG simultaneously, Group 7: Sodium arsenite (2 weeks) followed by 250mg/kg body weight IG (2 weeks). Group 8: Sodium arsenite (for 2 weeks) followed by 500mg/kg body weight IG (2 weeks). #= the mean difference is significant (p< 0.05) different when compared with control (group 1). *= the mean difference is significant (p< 0.05) when compared with the group given sodium arsenite only (group 2).

bone marrow cells (Figure 2) is significantly higher ($p < 0.05$) in the groups of rats administered sodium arsenite (groups 2) when compared with the negative control given only distilled water (group 1). Treatments with the extract caused a significant ($p<0.05$) reduction in the number of mPCEs scored in the bone marrow cells in the groups administered both the extract and sodium arsenite when compared with the group given sodium arsenite only. When compared with the positive control, given sodium arsenite only, the extract was able to significantly ($p<0.05$) reduce the number of mPCEs both when it was given along with sodium arsenite (groups 5 and 6) or given two weeks after sodium arsenite (groups 7 and 8).

Table 4. Phytochemical constituents of the ethanol extract of *Irvingia gabonensis* leaves.

Phytochemicals	Value
Tannins (mg/100g)	1431.7
Alkaloids (mg/100g)	33.3
Flavonoids(mg/100g)	663.3
Saponins (mg/100g)	23.0
Total phenols (GAE/100g)	85.0
Terpenoids (mg/100g)	13.0

GAE= Gallic Acid Equivalent (conventional units for phenols)

Effect of *Irvingia gabonensis* on intracellular levels of generated hydrogen peroxide (H_2O_2), catalase (CAT) and superoxide dismutase (SOD) activities in liver of the rats treated with sodium arsenite (SA).

There was significant ($p<0.05$) high level H_2O_2 CAT activity in the group of rats treated with SA (group 2) compared with the negative control group 1 (Table 3). Treatment with extract resulted in reduced generation of hydrogen peroxide (H_2O_2) and significant ($p<0.05$) difference in the CAT and SOD activities when comparisons were made between the groups treated with both SA and extract and the group administered SA alone.

Phytochemical analysis of ethanol extract of *Irvingia gabonensis* leaves.

Phytochemicals present in the ethanol extract of the leaves of *Irvingia gabonensis* in significant amounts are tannins, alkaloids, favonoids, saponins, polyphenols and terpenoids (Table 4). Tannin has the highest value of 1431.7 mg/100g followed by flavonoids (663.3 mg/100g). Tannin is more than double the value of flavonoids. Terpenoids are present in the least amount.

DISCUSSION

Arsenicals especially the trivalent forms are highly recognized as potent environmental toxicants (Abernathy *et al.,* 1999). Exposure to arsenic via the intake of contaminated water has been linked with diverse heath defects like certain forms of cancer, skin lesions, and non-cancer health effects such as

neurological disorders and impaired cognitive development in children (Abernathy *et al.*, 1999).

The liver is an important target organ for arsenic toxicity (Parvez *et al.*, 2006). Arsenic intoxication in experimental animals has been linked with micronucleus formation and hepatic tumors (Moore and Smith, 1997; Mazumder, 2005). This study examined the effect of the ethanol leaf extract of *Irvingia gabonensis* (Aubry-Lecompte ex O'RorkeBaill) in sodium arsenite model of hepatotoxicity and clastogenicity in Wistar rats.

Administration of sodium arsenite alone for four weeks produced significantly ($p<0.05$) lower change in percentage body weights compared with the negative control group. This suggests that sodium arsenite interfered with systemic activities in the body of the rats. The extract was able to reverse the above effect of sodium arsenite on growth of the experimental rats.

Aspartate aminotransferase (AST) and Alanine aminotransferase (AST) are members of transaminase family of enzymes. They are also known as aminotransferases, they catalyze the transfer of amino groups between L-alanine and glutamate for physiological purpose. ALT and AST are found in large amount in the liver and also small amount are found in the heart, kidney and muscles. When the liver is injured or inflamed as the case may be via its exposure to various forms of toxic substances, the level of ALT and AST in the blood is usually elevated. The level of these enzymes in the blood is directly related to the extent of the tissue damage (Lum and Gambino, 1972).

Gamma-glutamyltransferase (γGT) is the enzyme actively responsible for the extracellular catabolism of glutathione, in the main mammalian cells (Huseby, 1978). γGT is present on the outer surface of plasma membrane of most cell type and in blood, where it has been shown to form complexes with several plasma components particularly with albumin and lipoprotein (Huseby, 1982). The determination of serum γGT activity is well established diagnostic test for hepatobiliary diseases, and is used as a sensitive marker of liver damage. Elevated serum γGT activity is associated with diseases of the liver, biliary system and pancreas (Betro *et al.*, 1973; Huseby, 1982).

The results obtained from the assessment of the serum levels of AST, ALT and γGT indicate that sodium arsenite significantly ($p<0.05$) induced these serum enzyme activities as compared with the control. Increased gamma glutamyltransferase activity has been linked with hepatotoxicity, oxidative stress and chromosomal aberrations in cells (Lum and Gambino, 1972; Dinari *et al.*, 1979; Karmaker *et al.*, 1999). The observations made here are consistent with the findings from previous findings (Mallick *et al.*, 2003; Odunola, 2003; Odunola *et al.*, 2007). The hepatoprotective effect of the extract was independent of whether the extract is administered at the same time with sodium arsenite or two weeks after sodium arsenite. This indicates that the effect could be preventive or curative. Histopathological analysis of liver organs showed that there was no visible lesion in the liver organs of the animals in the control group and in the groups that were treated with sodium arsenite and leaf extract simultaneously (Groups 5 and 6) also in the groups that were treated with extract only (Groups 3 and 4). In the group that was treated with sodium arsenite only (group 2), there was a marked widespread thinning of hepatic cords which indicates the necrotic cell death of the hepatocytes. This is consistent with earlier reports on the hepatotoxicity of sodium arsenite (Odunola *et al.*, 2008; Gbadegesin *et al.*, 2009). There was multifocal centrilobular thining of the hepatic cords in the group that was post-treated with 250 mg/kg body weight (Group 7). There was also moderate kupffer cell hyperplasia in the liver organs of the animals in the group that was post-treated with 500 mg/kg body weight of extract (Group 8).

The relative number of micronucleated polychromatic erythrocytes scored in the rat bone marrow cells show that sodium arsenite significantly ($p<0.05$) induced the formation of micronuclei in the polychromatic erythrocytes of the rat bone marrow cells. This clastogenic effect of sodium arsenite is in line with previous reported findings (Mallick *et al.*, 2003; Odunola, 2003; Odunola *et al.*, 2007). There were also significant decrease ($p<0.05$) in the number of micronuclei scored in the groups treated with the leaf extract (250 mg/kg body weight and 500 mg/kg body weight respectively) after treatment with sodium arsenite, when compared with the group treated with sodium arsenite only. The observed decreased numbers of micronuclei in these groups indicate that the extract is anticlastogenic.

Findings from the present study supported the reports that sodium arsenite generate free radicals like H_2O_2 in living systems (Ramanathan *et al.*, 2002; Usoh *et al.*, 2005). Endogenous enzymes constitute the first line of cellular defense and provide a mutually supportive team of defence against reactive oxygen species (Ramanathan *et al.*, 2002). In sodium arsenite induced hepatotoxicity, the balance between the production of reactive oxygen species and these antioxidant defence systems may be lost, thus leading to oxidative stress due to the overwhelming power of prooxidants generated, which through a series of events deregulates the cellular functions that leads to hepatic necrosis (Amresh *et al.*, 2007). Catalase is responsible for the degradation of hydrogen peroxide which is a reactive oxygen species produced during metabolism. Catalase catalyzes the removal of

hydrogen peroxide formed during the reaction catalyzed by superoxide dismutase (Manna *et al*., 2007). Superoxide dismutase is an enzyme that protects against the superoxide radical which can cause oxidative stress. SOD speeds up the dismutation of superoxide radical to hydrogen peroxide which is then removed by catalase (Usoh *et al.*, 2005). SOD can therefore function as a primary defense and inhibits further production of free radicals. The increased amount of hydrogen peroxide, and therefore total reactive oxygen species, generated when rats were treated with sodium arsenite demonstrates treatment of experimental animals with sodium arsenite would contribute to oxidative stress. It is possible that the extract enhanced the antioxidant system in the treated rats therefore speeding up the mopping up of the reactive oxygen species.

From the results obtained for catalase (CAT) activity and superoxide dismutase (SOD) activity, the catalase activity was significantly ($p<0.05$) induced in the group treated with sodium arsenite only when compared with the control. The explanation to this may be that catalase activity was significantly induced as an adaptive response to the increased amount of hydrogen peroxide generated in the group as catalase is known to mop up hydrogen peroxide which is a reactive oxygen species. There was however no significant differences in catalase activities in the groups treated simultaneously with both sodium arsenite and extract suggesting that an immediate mopping up of the reactive oxygen species by the extract enhanced antioxidant system is inefficient. However, treatment with the extract, two weeks after sodium arsenite administration resulted in CAT activities that are significantly different from sodium arsenite treated group. Similar trends were observed form SOD activities across all the groups.

The result obtained from the phytochemical analysis of ethanol extract of *Irvingia gabonensis* (Aubry-Lecompte ex O'RorkeBaill) leaves indicated the presence significant amount of tannins, saponins, alkaloids, terpenoids, flavonoids and phenols. Tannin is more than double the value of flavonoids. Tannin has been reported to have anti-inflammatory and antiulcer property in rodents, showing a strong antioxidant property (Souza *et al*., 2006). In conclusion, ethanol extract of leaves of *Irvingia gabonensis* (Aubry-Lecompte ex O'RorkeBaill) showed potent hepatoprotective and anticlastogenic activities in sodium arsenite induced toxicity in rats. The extract also enhanced the enzymatic antioxidant status of the treated rats. These biological activities may be due to the phytochemicals present in the extract. Further studies to isolate, purify and characterise the active compounds in the extract are significant.

REFERENCES

Abernathy, C. O., Liu, Y. P., Longfellow, D., Aposhian, H. V., Beck, B., Fowler, B., Goyer, R., Menzer, R., Rossman, T., Thompson, C. &Waalkes, M. (1999). Arsenic: health effects, mechanisms of actions, and research issues. *Environ Health Perspect* 107(7): 593-597.

Adamson, I., Okafor, C., Abu-Bakare, A (1990). A supplement of Dikanut (Irvingia gabonesis) improves treatment of type II diabetics. *West Afr J Med*.9(2):108-115.

Ames, B. N. (1979). Identifying environmental chemicals causing mutations and cancer. *Science* 204(4393): 587-593.

Amresh, G., Rao, C. V. &Singh, P. N. (2007). Antioxidant activity of Cissampelos pareira on benzo(a)pyrene-induced mucosal injury in mice. *Nutrition research (New York)* 27(10): 625-632.

Betro, M. G., Oon, R. C. &Edwards, J. B. (1973). Gamma-glutamyl transpeptidase and other liver function tests in myocardial infarction and heart failure. *Am J Clin Pathol* 60(5): 679-683.

Dinari, G., Cohen, M. I., McNamara, H. &Kochen, J. A. (1979). Effect of lead exposure on the activity of some hepatic enzymes in the rat. *Pediatr Res* 13(5 Pt 1): 644-646.

Gbadegesin, M. A., Odunola, O. A., Akinwumi, K. A. &Osifeso, O. O. (2009). Comparative hepatotoxicity and clastogenicity of sodium arsenite and three petroleum products in experimental Swiss Albino Mice: the modulatory effects of Aloe vera gel. *Food Chem Toxicol* 47(10): 2454-2457.

Guillamet, E., Creus, A., Ponti, J., Sabbioni, E., Fortaner, S. &Marcos, R. (2004). In vitro DNA damage by arsenic compounds in a human lymphoblastoid cell line (TK6) assessed by the alkaline Comet assay. *Mutagenesis* 19(2): 129-135.

Hubert, D.J., Wabo, F.G.,Ngameni, B., Ngheguin, T.F., Tchoukoua, A., Ambassa, P., Abia, W., Tchana, A.N., Giardina, S., Buonocore, D., Vita, F.P., Vidari, G., Marzatico, F., Ngadjui, B.T., Moundipa, P.F. (2010). "*In vitro* hepatoprotective and antioxidant activities of the crude extract and isolated compounds from *Irvingia gabonensis*. *Asian Journal of Traditional Medicine*. 5(3): 79-88

Huseby, N. E. (1978). Multiple forms of gamma-glutamyltransferase in normal human liver, bile and serum. *Biochim Biophys Acta* 522(2): 354-362.

Huseby, N. E. (1982). Multiple forms of serum gamma-glutamyltransferase. Association of the enzyme with lipoproteins. *Clin Chim Acta* 124(1): 103-112.

Karmaker, R., Banerjee, A., Datta, S., and Chatterjee, M. (1999). Influence of cadmium intoxification on hepatic lipid peroxidation, glutathione level and glutathioneS-transferase and gamma-glutamyl transpeptidase activities: Correlation with chromosome aberration in bone marrow cells. *J. Environ. Pathol. Toxicol. Oncol.* 18: 277-287.

Lee, D. H., Blomhoff, R. &Jacobs, D. R., Jr. (2004). Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radic Res* 38(6): 535-539.

Lum, G. &Gambino, S. R. (1972). Serum gamma-glutamyl transpeptidase activity as an indicator of disease of liver, pancreas, or bone. *Clin Chem* 18(4): 358-362.

Mallick, P., Mallick, J. C., Guha, B. &Khuda-Bukhsh, A. R. (2003). Ameliorating effect of microdoses of a potentized homeopathic drug, Arsenicum Album, on arsenic-induced toxicity in mice. *BMC Complement Altern Med* 3: 7.

Manna, P., Sinha, M. &Sil, P. C. (2007). A 43 kD protein isolated from the herb Cajanus indicus L attenuates sodium fluoride-induced hepatic and renal disorders in vivo. *J Biochem Mol Biol* 40(3): 382-395.

Matos L, Nzikou JM, Matouba E, Pandzou-Yembe VN, Mapepoulou TG, Linder M, Desobry S (2009). Studies of *Irvingia gabonensis* seeds kernels: Oil technological applications. *Pak. J, Nutr.* 8: 151-157

Matter, B. &Schmid, W. (1971). Trenimon-induced chromosomal damage in bone-marrow cells of six mammalian species, evaluated by the micronucleus test. *Mutat Res* 12(4): 417-425.

Mazumder, D. N. (2005). Effect of chronic intake of arsenic-contaminated water on liver. *Toxicol Appl Pharmacol* 206(2): 169-175.

Moore, L. E., Smith, A. H., Hopenhayn-Rich, C., Biggs, M. L., Kalman, D. A. &Smith, M. T. (1997). Decrease in bladder cell micronucleus prevalence after intervention to lower the concentration of arsenic in drinking water. *Cancer Epidemiol Biomarkers Prev* 6(12): 1051-1056.

NAERLS (National Agricultural Extension and Research Liaison Services) (1999) Production and utilization of Ogbono *(Irvingia gabonensis).* Extension bulletin No 140. Horticulture series No 4. Pp. 3-12.

Nosiri, I., Abdu-Aguye, I., Hussaini, I. &Abdurahaman, E. (2009a). Leaf Extracts of *Irvingia gabonensis* Increase Urine Output And Electrolytes In Rats. *The Internet Journal of Alternative Medicine* 8(2): http://ispub.com/IJAM/8/2/11789. Accessed June 2014.

Nosiri, I., Hussaini, I., Abdu-Aguye, I. &Abdurahaman, E. (2009b). Pharmacological Effect of Irvingia gabonensis Leaf Extracts on Cat Blood Pressure. *The Internet Journal of Pharmacology* 9(1): http://ispub.com/IJPHARM/9/1/6633. Accessed June 2014.

Odunola, O. A. (2003). Comparative effects of some local food condiments on sodium arsenite-induced clastogenicity. *Afr J Med Med Sci* 32(1): 75-80.

Odunola, O. A., Akinwumi, K. A., Ogunbiyi, B. &Tugbobo, O. (2007). Interaction and Enhancement of the Toxic Effects of Sodium Arsenite and Lead Acetate in Wistar Rats. *African Journal of Biomedical Research* 10: 59 - 65

Odunola, O. A., Uka, E., Akinwumi, K. A., Gbadegesin, M. A., Osifeso, O. O. &Ibegbu, M. D. (2008). Exposure of laboratory mice to domestic cooking gas: implications for toxicity. *Int J Environ Res Public Health* 5(3): 172-176.

Okolo, C. O., Johnson, P. B., Abdurahman, E. M., Abdu-Aguye, I. &Hussaini, I. M. (1995). Analgesic effect of Irvingia gabonensis stem bark extract. *J Ethnopharmacol* 45(2): 125-129.

Osadebe, P. O., Abba, C. C. &Agbo, M. O. (2012). Antimotility effects of extracts and fractions of Eastern Nigeria mistletoe (Loranthus micranthus Linn). *Asian Pac J Trop Med* 5(7): 556-560.

Parvez, F., Chen, Y., Argos, M., Hussain, A. Z., Momotaj, H., Dhar, R., van Geen, A., Graziano, J. H. &Ahsan, H. (2006). Prevalence of arsenic exposure from drinking water and awareness of its health risks in a Bangladeshi population: results from a large population-based study. *Environ Health Perspect* 114(3): 355-359.

Perera, F. P., Mooney, L. A., Stampfer, M., Phillips, D. H., Bell, D. A., Rundle, A., Cho, S., Tsai, W. Y., Ma, J., Blackwood, A. &Tang, D. (2002). Associations between carcinogen-DNA damage, glutathione S-transferase genotypes, and risk of lung cancer in the prospective Physicians' Health Cohort Study. *Carcinogenesis* 23(10): 1641-1646.

Preston, R. J., Dean, B. J., Galloway, S., Holden, H., McFee, A. F. &Shelby, M. (1987). Mammalian in vivo cytogenetic assays. Analysis of chromosome aberrations in bone marrow cells. *Mutat Res* 189(2): 157-165.

Ramanathan, K., Balakumar, B. S. &Panneerselvam, C. (2002). Effects of ascorbic acid and alpha-tocopherol on arsenic-induced oxidative stress. *Hum Exp Toxicol* 21(12): 675-680.

Reitman, S. &Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 28(1): 56-63.

Souza, S. M. C., Aquino, L. C. M., Jr, A. C. M., Bandeira, M. A. M., Nobre, M. E. P. &Viana, G. S. B. (2007). Antiinflammatory and antiulcer properties of tannins from Myracrodruon urundeuva Allemão (Anacardiaceae) in Rodents. *Phytotherapy Research* 21(3): 220-225.

Szasz, G. (1969). A kinetic photometric method for serum gamma-glutamyl transpeptidase. *Clin Chem* 15(2): 124-136.

Usoh, I. F., Akpan, E. J., Etim, E. O. &Farombi, E. O. (2005). Antioxidant actions of dried flower extracts of Hibiscus sabdariffa L. on sodium arsenite-induced oxidative stress in rats. *Pak. J. Nutr.* 4: 135-141.

Niger. J. Physiol. Sci. 29(June 2014) 037 –042
www.njps.com.ng

Niger. J. Physiol. Sci.

The role of oropharnygeal receptors in thirst perception after dehydration and rehydration

*** Obika, LFO., Okpere, S.O., Ozoene, J.O. and Amabebe, E.**

Department of Physiology, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

Summary: This study examined the effect of drinking and gargling on thirst perception (TP) in 33 young dehydrated female subjects (18-25yrs), using the visual analogue scale (VAS). Group A subjects drank, while group B gargled the fluid provided - 0.0%, 0.9% and 1.8% NaCl (7.0 ml/kg body weight of fluid). The procedure was alternated two weeks later. All subjects dehydrated for 18 hours prior to the study, and the last 12-hour urine was collected and volume recorded. Subject who provided a 12hr urine volume greater than 400ml was excluded from the study. After recording the baseline TP, and voiding the bladder, drinking/gargling was done within 5 minutes, and the subsequent TPs were recorded at 5 minutes interval for 25 minutes. Blood samples were collected before and at the end of the 30 minutes, when urine volumes were recorded. Drinking (0.0% and 1.8% NaCl) resulted in an initial decrease in thirst perception, which was statistically significant ($p<0.05$) only up to 10 minutes. Water intake ad libitum (mean ± SEM) at the end of the 30 minutes was statistically significantly lower ($p<0.05$) only in the group that drank 0.0% NaCl. Gargling on the other hand did not affect TP and water intake throughout the period of study. It can be concluded that drinking, but not gargling reduces thirst perception irrespective of the tonicity of the fluid as earlier reported (Obika et. al., 2009; Salata et. al., 1987). This study suggests that the oropharyngeal receptors for TP are activated by recurrent stimulation by the act of drinking rather than gargling.

Keywords*:* Dehydration, Rehydration, Oropharyngeal Receptors, Thirst perception, Drinking, Gargling.

**Address for correspondence:* lfoozy@yahoo.com

Manuscript Accepted: April, 2014

INTRODUCTION

Thirst is a conscious sensation of a need for water (Robertson, 1991). It is important for maintaining body fluid homeostasis and may arise from deficits in either intracellular or extracellular fluid volume (Micheal et al., 2004). The stimuli for thirst includes increase in plasma osmolality, decrease in blood volume, decrease in blood pressure, increase in angiotensin II, dryness of mouth and throat (Adolph et al., 1954). Among the above listed stimuli, evaluation of changes in the concentration of blood extracellular fluid, measured as the osmolality of blood plasma appears to be the primary regulator of thirst.

Water deprivation results in increased plasma osmolality, thirst and increased secretion of arginine vasopressin, AVP (Bayliss and Robertson, 1980Stricker and Verbalis, 2002). Plasma osmolality has been linked inextricably to determine the sensation of thirst (Baylis and Robertson, 1980). In humans, thirst and AVP are controlled by similar sensitive osmoregulatory mechanisms such that above a certain osmotic threshold of 280 – 288 mOsm/kg H_2O, there is a linear relationship between the increase in plasma osmolality (P_{osm}) and the increase in AVP and thirst (Bayliss and Robertson, 1980). Robertson (1991) determined the osmotic threshold for the onset of thirst to be about 294mOsm/kg H_2O while that of vasopressin release threshold to be about 284 mOsm/kg H_2O in healthy humans.

Geelen et al., (1984) reported that the close relationship between P_{osm} and P_{AVP} is lost during the act of drinking which causes rapid suppression of vasopressin secretion before changes in P_{osm} occurs. It has also been documented in dogs and humans (Bruner, 1993; Haung et al., 2000; Obika et al., 2009) that when dehydrated animals drink, vasopressin secretion and thirst is rapidly inhibited before systemic rehydration is evident. Because absorption of ingested water must take time, and systemic factors do not change rapidly enough to account for the termination of drinking in many species, some other rapid, perhaps preabsorptive factor or factors must be important. One of such possible mechanism could arise from the oropharyngeal stimulation which occurs while water is being ingested. Simply tasting

the water and swallowing it could be sufficient to induce satiety and therefore terminate drinking (Obika et al., 2009).

The early signal that inhibits thirst has been widely related to oropharyngeal receptors signals originating from the oropharyngeal region (Figora and Mack, 1997). Oropharyngeal receptors not only contribute to the sensation of thirst but also to the control of vasopressin secretion (Geelen et al., 1984). Although oropharyngeal factors acting alone may not be sufficient to account for the normal termination of drinking in most species, it has however been suggested to play a role in the initiation and maintenance of drinking as well as to contribute to the termination of drinking (Holmes, 1964; Rolls et al., 1980; Applegreen et al., 1991; Ajayi and Obika, 2000).

Research is still on going to fully determine the definitive role of oropharyngeal receptors. Obika et al. (2009) reported a reduction in thirst during sham-drinking experiments involving the stimulation of the oropharyngeal receptors by repeated gargling in man. Since drinking involves "repeated gargling", the purpose of this work is to determine the effect of drinking and "single gargling" on thirst perception in young dehydrated female subjects.

MATERIALS AND METHODS

Subjects

The subjects were thirty-three (33) apparently healthy female undergraduate volunteers, between the ages of 18 and 25 years, who gave their consent. Two Groups of studies A and B were carried out and each subject participated in the two studies, separated by a two (2) week interval. The subjects in Group A drank while those in Group B did not drink (i.e., gargled) the 0.0%, 0.9% and 1.8% NaCl solutions. For this study the subjects were further divided into three sub-groups which they maintained for both studies. Subgroup 1 of either Group A or B drank or gargled distilled water (0.0% NaCl solution) respectively. Similarly, subgroups 2 and 3 drank or gargled 0.9% or 1.8% NaCl solution respectively.

Procedure for dehydration

All subjects abstained from drinking water or any other fluid for 18hours (3pm-9am). Their last 12hours urine (9pm-9am) was collected in a container and the volume recorded. Subjects with urine volume greater than 400ml were considered not to have adhered to the dehydration procedure and were therefore excluded from the study.

Each of the subjects were dehydrated and arrived at the laboratory on the day of the experiment prior to the end of the dehydration period. Resting blood pressure was measured after 15 minutes of rest in the laboratory with the subject seated and the right hand supported at heart level. Two (2) basal readings were obtained on each subject at 3 minutes interval and the mean was recorded as the normal blood pressure.

The subject's anthropometric data were taken and body mass index was calculated from-

$$BMI = \frac{Weight\ (kg)}{height\ (cm)}$$

At the end of the dehydration period (9am) final urine sample was collected to make up the 12hours urine volume (9pm - 9am). Aliquots of the urine were analyzed for Na^+, K^+, Cr and Urea conentrations. Blood samples were collected from each of the subjects and kept in appropriate anticoagulant bottles which were properly labeled. After centrifugation, the plasma concentrations of Na^+, K^+, Cr and Urea were determined. Baseline thirst ratings were estimated by the subjects, using the Visual Analogue Scale (VAS) as modified by Thompson et al., (1991). The Visual Analogue Scale (VAS) is a 10cm marked vertical but uncaliberated line which is labeled "**Very thirsty"** at the top mark and "**Not thirsty"** at the bottom mark. The measurement from the bottom mark, to the mark made on the VAS by the subject in response to the question "**How thirsty are you NOW?"** gives a subjective rating of the thirst perception (TP) at that point in time.

Group A Study: Effect of drinking distilled water and NaCl solutions on thirst perception

Thereafter, the subjects drank the respective solutions provided (7.0ml/kg body weight of 0.0%, 0.9%, or 1.8% NaCl) within 5mins. TP was again measured using the VAS after drinking and at five (5) minutes interval for the next thirty (30) minutes. At the 30^{th} minute, urine samples were collected in separate bottles which made up 30 minutes urine volume, blood samples were once again collected.

Finally the subjects were provided with bottles of distilled water with volumes unknown to them. They were asked to drink freely till satiety and the volume of water drunk by each subject was calculated by subtracting the final volume of fluid in the container from the initial volume.

The subjects from group A study returned to the laboratory after a 2 weeks interval for the group B study, both studies were carried out on the same individuals to avoid large error margins.

Group B study: Effect of gargling distilled water and NaCl solutions on thirst perception

As in Group A study, subjects again went through an 18 hour dehydration period. The subjects then gargled the respective solutions (7.0ml/kg body weight of 0.0%, 0.9% or 1.8 % NaCl). They gargled the entire volume comfortably within 5 minutes, and returned the already gargled fluid continuously into a container provided. Thereafter, and at 5 minutes interval and for 30 minutes, TP was measured using the VAS. Blood samples were again collected as well as 30 mins urine samples from each subject.

Finally, subjects drank ad-libitum the distilled water provided and the volume drunk was calculated as earlier stated. The gargled fluid returned to the containers were measured and subjects who returned volumes significantly lower than the initial volume given to them were excluded from the experiment.

Calculations:

1. Fractional excretion of water, (FE_{H20}) was calculated using the formula:

$$FEH2O = \frac{Pcr\ (\frac{mg}{dl})}{Ucr\ (mg/dl)}$$

Where Pcr =Plasma creatinine concentration
Ucr =Concentration of creatinine in urine.

2. Fractional excretion of sodium, $FENa^{+}$ was calculated using the formula:

$$FENa^{+} = \frac{UNa\ (mol/(l)\ x\ PCr(mg/dl)}{PNa\ (mol/(l)\ \ x\ UCr\ (mg/dl)}$$

Where UNa = Urinary sodium concentration
PNa = Plasma sodium concentration
PCr = Plasma creatinine concentration
UCr = Concentration of creatinine in Urine.

Statistical analysis:
Data were presented as mean ± standard error of mean (SEM). Intra group and inter group comparisons were made using the one way analysis of variance (ANOVA). The Students't-test was used for comparisons between the experimental and control values. Confidence limit was set at 95% and a P value less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows the anthropometric data and baseline readings after dehydration in groups A and B subjects. There was no significant difference in these values measured two weeks apart in the subjects. Table 2 shows the mean values of some urine characteristics, which were not statistically significant between the two groups of subjects

Group A: **Effect of drinking 0.0%, 0.9% and 1.8% NaCl solution on thirst perception**.
The baseline thirst rating in group A study and their thirst ratings after drinking are as shown figure 1. The control TP value (Baseline TP) was not significantly different within the subgroups. There was a decrease ($p<0.05$) in TP in all the subgroups within five minutes after drinking, irrespective of the solution drank, although this decrease was only significant in subjects that drank 0.0% and 1.8% NaCl solution. The gradual decrease in mean TP continued significantly up to the 15 minutes in the subjects that drank 0.0%. These decreases in TP were followed by a gradual rise to the baseline values at the end of the experiment. On the other hand, in the 1.8% NaCl subgroup, the significant fall in TP was followed by a significant rise in the 10th minute after drinking. The TP continued to rise till the termination of the experiment.

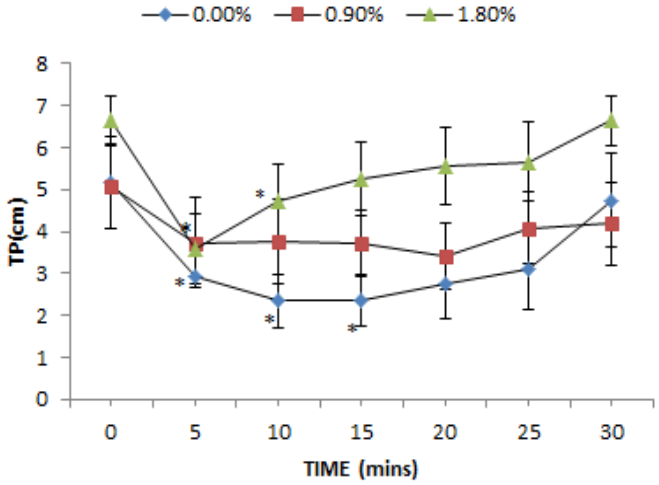

Figure 1: shows the mean basal TP (cm) in the dehydrated female subjects (TP at 0), and the changes in the mean TP at 5mins interval for 30mins, after drinking 0.0%, 0.90% or 1.8% NaCl within the first 5mins. * =$p<0.05$.

Group B: Effect of gargling 0.0%, 0.9% and 1.8% NaCl solution on thirst perception.
TP was recorded in these subjects at 0mins (baseline TP) and at 5 minutes interval for 30 minutes after gargling. The control TP (baseline) was not significantly different within the subgroup, and there was no significant change in TP within 30 minute after gargling with 0.0%, 0.9% and 1.8% NaCL. This is in marked contrast to the observation in the group that drank the fluids.

Table 1: Anthropometric data, Blood pressure indices and serum Na, K, Cr and Urea level collected from the subjects on arrival at the laboratory (expressed as mean ± SEM).

Group	Age (yr)	SBP(mmHg)	DBP(mmHg)	Wt (Kg)	Ht (cm)	BMI(Kg/m^2)	Na^{+}(mmol/L)	K^{+}(mmol/L)	Cr (mmol/L)	Urea (mmol/L)
A (n=33)	20.94±0.35	111.5±0.5	63.94±0.39	59.94±0.64	162.7±0.4	23.04±0.26	135.0±0.2	3.65±0.04	0.67±0.01	10.71 ±0.22
B (n=33)	20.94±0.35	112.1±1.04	64.55 ±0.88	60.24±1.83	162.7±1.03	23.15±0.73	134.9±0.71	3.62±0.12	0.69±0.04	10.90±1.63

Table 2: Mean baseline characteristics of 12hr urine samples collected during the dehydration period from the subjects studied (expressed as mean ± SEM).

Group	Volume (ml)	flow rate (ml/min)	Na^{+} (mmol/L)	K^{+} (mmol/L)	Cr (mmol/L)	Urea (mmol/L)	FE Na^{+}	FE H_2O
A (n=33)	353.12 ±22.29	0.49±0.03	201.86 ±9.46	48.20 ±4.91	16.16 ±0.73	396.36 ±12.67	4.16 ±0.29	3.55 ±0.77
B (n=33)	345.52±24.54	0.49±0.03	197.86 ±6.02	45.88 ±7.64	14.68 ±0.23	399.19 ±9.84	4.99 ±O.22	3.80 ±0.10

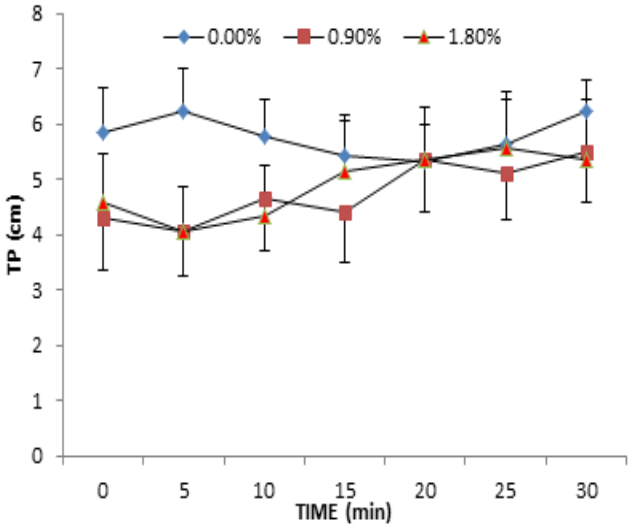

Figure 2: Shows the mean basal TP (cm) in the dehydrated female subjects (TP at 0), and the changes in the mean TP at 5mins interval for 30mins, after gargling with 0.0%, 0.90% or 1.8% NaCl within the first 5mins.

Effect of drinking/gargling the same concentration of NaCl solution on TP

The graph shows the effect or drinking and gargling the same volume of 0.0% (Fig. 3), 0.9% (Fig. 4) and 1.8% (Fig. 5) NaCl solution on thirst perception in the same dehydrated subjects at two weeks interval. As shown in Fig. 3, TP was statistically significantly lower in DW in group A than GW in group B, from the 5th minute to the 25th minute after drinking/gargling the same volume of distilled water. There was however no statistical significant change in TP with gargling and/or drinking of the same volume of 0.9% NaCl (Fig.4) and 1.8% NaCl (Fig. 5) in the dehydrated subjects as shown in the graphs.

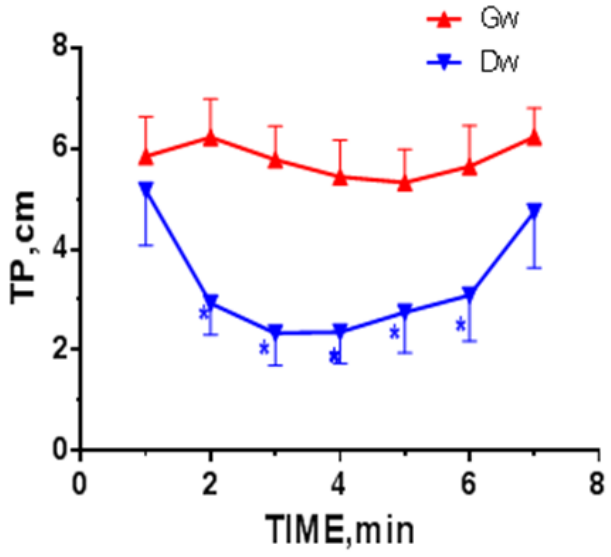

Figure 3: Effect of drinking/gargling of 0.0% NaCl solution on TP. * =p<0.05

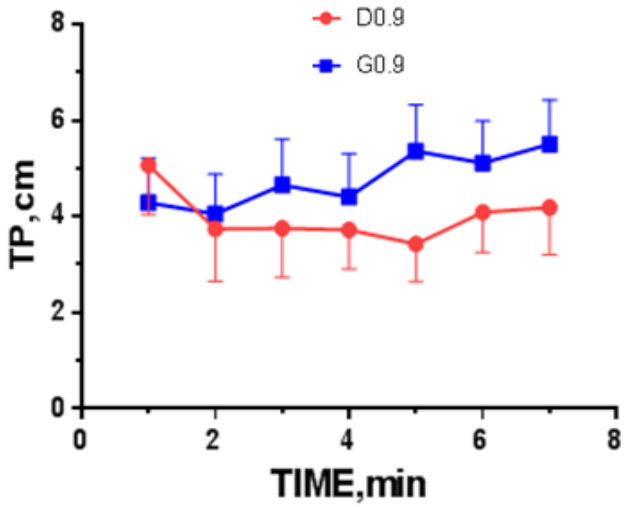

Figure 4: Effect of drinking/gargling of 0.9% NaCl solution on TP.

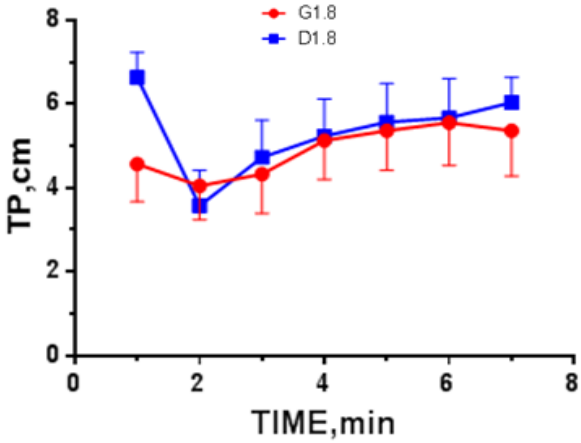

Figure 5: Effect of drinking/gargling of 1.8% NaCl solution on TP. * =p<0.05

Other parameters

Other parameters including those calculated from results of urine and blood sample analysis are as shown in tables 3 and 4. All the parameters measured in this Group A were similar within the subgroups, except the *ad libitum* water intake, which was significantly ($p<0.05$) lower in the subgroup that drank water.

The volumes gargled and that recovered were similar in Group B (Table 4), indicating that the subjects in this group did not drink the solution. There was no significant difference between the subgroups in all the other parameters measured in Group B.

When Groups A and B were compared, there was no difference in the *ad libitum* water intakes (except in those that drank 0.0% NaCl solution: 63.6±35.9 vs 263.2±45.4ml, $p<0.05$). The volume of 12hr urine output and the fractional excretion of water were generally lower in Group B, though not statistically significant.

Table 3: Shows the volume of fluids drunk, 12 hour urine volume, fluid intake ad-libitum, and 30 mins urine volume, fractional excretion of water and fractional excretion of sodium after drinking in Group A.

	Vol. of fluid intake (7.0ml/kg)	Regurgitated Vol. (ml)	Volume Of fluid Intake *ad libitum* (ml)	30 mins Urine Vol. (ml)	12hr Urine Vol.	FE_{H2O}	FE_{Na+}
D.WATER (N=11)	434.36±29.14	0	*****63.63±35.93	52.85±7.87	307.36±52.95	6.14±1.90	5.80±0.29
0.9% NaCl (N=10)	408.10±18.69	0	243.45±70.22	48.50±17.02	326.5±30.90	5.94±1.77	6.09±0.36
1.8% NaCl (N=12)	417.25±18.82	0	279.17±40.04	47.12±12.18	396.33±36.44	5.09±1.13	6.46±0.27

*(***=** $P \leq 0.05$), vs Euhydrate and Dehydrate*

Table 4: Shows the volume of fluids gargled, 12 hour urine volume, fluid intake ad-libitum and 30mins urine volume, fractional excretion of water and fractional excretion of sodium after gargling in Group B.

	Vol. of fluid intake (7.0ml/kg)	Regurgitated Vol. (ml)	Volume Of fluid Intake *ad libitum* (ml)	30 mins Urine Vol. (ml)	12hr Urine Vol.	FE_{H2O}	FE_{Na+}
D.WATER (N=11)	438.45±28.37	438.27±27.90	263.18±45.40	28.08±17.71	322.09±32.41	4.16±0.29	5.80±0.29
0.9% NaCl (N=10)	411.3±18.94	405.30±21.15	291.0±64.85	22.80±4.35	359.80±37.51	4.15±0.23	6.09±0.36
1.8% NaCl (N=12)	416.50±19.35	413.25±19.46	264.08±38.59	24.58±4.01	291.58±28.98	4.23±0.18	6.46±0.27

DISCUSSION

The visual analogue scale has been widely used with success as an indirect tool in measuring thirst perception in individuals (Seckl et al, 1986; Obika et al., 2009). The thirst ratings obtained from the scale has been shown to be highly reproducible within individuals on repeated testing (Thompson *et al*., 1991). In this study the VAS was also employed in assessing thirst perception in dehydration and rehydration. The baseline thirst perception immediately before drinking or gargling the same volume of the solutions remained similar in the two groups of study.

From this study a gradual fall in the mean thirst rating was observed in all the dehydrated subjects that drank the solutions. This decrease was statistically significant with 0.0% and 1.8% NaCl solution. This decrease occurred right from the 5^{th} minute after drinking irrespective of the tonicity of the fluid drunk. The time duration within which these changes were observed is obviously not sufficient for substantial changes in plasma osmolality to have occurred through absorptive route (Obika et al., 2009). Since this decrease in TP occurred immediately after drinking in these subjects, the presence of fluid in the mouth and the act of swallowing may have actually stimulated the oropharyngeal receptors to cause the decrease in thirst perception (Holmes 1964; Applegreen et al., 1991; Igbokwe and Obika, 2008).

As observed above, drinking brought about a decrease in thirst perception irrespective of the tonicity of fluid drunk. In previous works by Obika and Mowoe (1997), and Rolls et al., (1980) the authors reported a fall TP in normal euhydrate subjects when hypertonic, and/or hypotonic fluid loads were administered. Geelen et al. (1984) also reported a decrease in thirst after drinking hypertonic saline. Salata et al. (1987) reported no changes in vasopressin level in subjects who held concentrated solutions in their mouths for 30mins and thus concluded that oropharyngeal receptors are not responsive to local changes in osmolality. This is in line with our findings that the tonicity of fluid is irrelevant with respect to the response of oropharyngeal receptors to thirst perception. In addition, Crammer (1991) concluded from his study that drinking is an activity determined partly by oropharyngeal stimulation, and that thirst need not be involved.

In the contrast, thirst ratings taken after "single gargling" in this experiment showed no significant change from the control throughout the duration. However, Obika et al. (2009), recorded a significant decrease in thirst perception upon "repeated gargling" at 10 minutes interval for 60 minutes with 0.0%, 0.9% and 1.8% NaCl solution, gargling was done for the first 5minutes within the 10 minutes interval, and thirst perception was rated 5minutes later, and thus they reported that the reduction in thirst perception by the oropharyngeal receptors was unrelated to drinking. Although single gargling showed no significant change throughout the duration of the experiment, from the above it can be suggested that the receptors present in the mouth require some form of agitations such as repeated gargling as was carried out by Obika et al. in 2009, or continuous intake of bouts of fluid as occurs in drinking, to produce a response, since acute stimulation ("single gargling") as carried out in this study did not bring about a significant change in thirst perception. These results suggest that the oropharyngeal receptors may be rapidly adapting.

Furthermore, the initial decrease in thirst perception after drinking was followed by a gradual rise in mean thirst rating towards the control TP by the 30^{th} minutes. Accordingly, the volume of fluid

drunk *ad-libitum* by the subjects at the end of the experiment was not significantly different after drinking or gargling of the fluids, but was only significantly lower in the subjects that drank 0.0% NaCl solution. This suggests that oropharyngeal stimulations results in the initial decrease in thirst, but does not abolish thirst.

It can be concluded from this study, that drinking but not "single gargling" reduces thirst perception irrespective of the tonicity of the fluid. This study further suggests that the oropharyngeal receptors are activated by recurrent stimulation by the act of drinking and may be rapidly adapting.

Part of this work has been presented at the 33rd Annual Scientific Conference of the Nigeria Physiological Society, held at the Department of Physiology of the University of Ibadan, Nigeria, 12-15th February, 2014.

REFERENCES

Ajayi, OI and Obika, LFO. (2000): Role of oropharyngeal receptors in thirst perception: Abstract No 54 of the 22nd Scientific Conference of the Physiological Society of Nigeria.

Appelgren, BH., Thrasher, TN., Keil, LC and Ramsay, DJ. (1991): Mechanism of drinking induced inhibition of Vasopressin secretion in dehydrated dogs. *Am. J. Physiol. Regulatory, Integrative Comp. Physiol*., 261: R1226-R1233.

Bayliss, PH and Robertson, GL. (1980): Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function. *J. Roy. Med.*, 73: 255-260.

Bruner, FP. (1993): Pathophysiology of dehydration. *Schweiz Rundsch. Med. Prax.* 82(29-30): 784-787.

Crammer, JL. (191): Drinking, thirst and water intoxication. *Br. J. Psychiatry.* 159: 83-89.

Figora, MK and Mack, GW. (1997): Regulation of fluid intake in dehydrated humans: role of oropharyngeal stimulation. *Am. J Physiol*., 272: 1740-1746.

Fitzsimons, JT. (1979): *The Physiology of Thirst and Sodium Appetite*. Cambridge, UK: Cambridge University Press. 129-222.

Geelen, GI., Keil, LC., Kravik, SE., Wade, CE., Thrasher, TN and Barnes, PR. (1984). Inhibition of plasma Vasopressin by drinking in healthy humans. *Am. J. Physiol*., 247: R247-R971.

Haung, W., Sved, AF and Striker, EM. (2000): Water injection provides an early signal inhibitin osmotically stimulated vasopressin secretion in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 279(3): R756-R760.

Holmes, JH. (1964): Thirst and fluid intake problems in clinical medicine in thirst. Ed: MJ. Waymer, NY. Macmillian. 345-364.

Igbokwe, VU and Obika, LFO. (2008): Thirst perception and dryness of mouth in healthy young adult Nigerians. *Afri. Biomed. Res*., II 39-46.

Obika, LFO, Idu, FK., George, GO., Ajayi, OI and Mowoe, R.S. (2009): Thirst perception and drinking in euhydrate and dehydrate human subjects. *Nig. J. Physiol. Sci.*, 24(1): 25-32.

Obika, LFO and Mowoe, RS. (1997): Thirst perception in young adult males before and after an oral fluid load and after dehydration. *International Union of Physiological Society Congress.* Abstract No. 101.01.16.

Robertson, GL. (1991): Disorders of thirst in Man. *In: Thirst, physiological and psychological aspects.* (eds: DJ. Ramsay and DA. Booth), Springer Verlag, London. Pp. 453-477.

Rolls, BJ., Wood, RJ., Rolls, ET., Lind, HM., Lind, RW and Ledingham, JGG. (1980): Thirst following water deprivation in humans. *Am. J. Physiol*., 239: R476-R482.

Salata, R.A., Verbalis, JG., Robinson AG. (1987): Cold water stimulation of oropharyngeal receptors in man inhibits release of vasopressin. *J. Clin. Endocrinol. Metab.*, 65(3): 561-7.

Seckl, JR., Williams, DM and Lightman, SL. (1986): Oral hypertonic saline causes transient fall in vasopressin in humans. *Am. J. Physiol.*, 251: R214-R217.

Stricker, EM and Verbalis, JG. (2002): Fluid intake and homeostasis. *In: Fundamental Neuroscience* (2nd ed.), edited by Squire, LR., Bloom, FE., McConnell, SK., Roberts, JL., Spitzer, NC and Zigmond MJ. Academic Press, San Diego, CA. Pp. 1011-1029.

Thompson, C.J., Selby, P and Bayliss, P.H. (1991): Reproducibility of osmotic and non-osmotic tests on vasopressin secretion in men. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., 260: 533-539

Zerbe, RL and Robertson, GL. (1983): Osmoregulation of thirst and vasopressin secretion in human subjects: effect of various solutes. *Am. J. Physiol. Endocrinol. Metab.*, 244: E607–E614.

Niger. J. Physiol. Sci. 29(June 2014) 043 –046
www.njps.com.ng

Niger. J. Physiol. Sci.

Histological changes in the cerebelli of adult wistar rats exposed to cigarette smoke

*** Gabriel Olaiya Omotoso and Farouq Abiodun Babalola**

Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

Summary: The different constituents of tobacco smoke have been linked to different diseased conditions. In this work, the histological effects of cigarette smoke on the cerebellum of adult male Wistar rats were studied. Sixteen Wistar rats with mean weight of 153.24 ± 4.12 g were grouped equally into four. The Control Group A was exposed to fresh air, while Groups B, C and D animals were each exposed to smoke from one, two and three sticks of cigarette respectively. Each stick of cigarette was completely consumed within an average duration of 11 minutes. Improvised smoking chambers were constructed and used for the exposure daily, while treatment lasted for 28 days. The animals were thereafter sacrificed by cervical dislocation, the cranium was exposed and the brain gently removed and weighed; the cerebellum was excised, weighed, and fixed in formol calcium, and subsequently processed for histological observation using the Haematoxylin and Eosin staining principle. Loss of weight and reduction in weight gain were noticed in the treatment groups, with corresponding reduction in cerebellar weights, in a dose-dependent pattern. Histology also revealed loss of white matter, reduction in thickness of cell layers and their cellular components. Increasing dosage of cigarette smoke could predispose to progressive compromise in the structural integrity and composition of the cerebellum, and this might result in cerebellar dysfunction.

Keywords*:* Cerebellar histology, cerebellar dysfunction, cigarette smoke, rats.

**Address for correspondence:* gabrielolaiya@yahoo.com Phone: +234703 050 5707

Manuscript Accepted: April, 2014

INTRODUCTION

The smoke of cigarette contains several chemicals that are potentially toxic and carcinogenic to the human body, and many of these constituents have been linked to different diseased states. Cigarette smoking is a known risk factor of many clinical conditions, and can also exacerbate some conditions (Henderson, 2008; Jill *et al*., 2006; Tatum and Shapiro, 2005). Since many of these constituents, like nicotine, cross the blood-brain barrier, the brain has become a target also of the toxic effects associated with tobacco smoke.

Studies on long-term neurotoxicity have observed increased formation of free radicals and oxidative stress in animals exposed to tobacco and alcohol *in utero* (Li and Wang, 2004). Carbon monoxide, a component of smoke, contributes to the hypoxic state of the brain. Prenatal exposure to carbon monoxide causes reduced birth weight and decreased weight gain in offspring of smokers, as well as lower behavioural activity levels, altered central catecholamine activity, and reduction in total brain protein at birth (Fechter and Annau, 1977). These go on through childhood to cause some forms of learning and memory deficits (Mactutus and Fechter, 1984). The high concentration of reactive oxygen species (ROS) in smoke is probably one of the major factors contributing to a high incidence of many clinical conditions linked with cigarette smoking (Palozza *et al*., 2006).

Chronic nicotine treatment induces CYP2E1 expression in the cortical pyramidal neurons and cerebellar Purkinje cells, and increased CYP2E1 in the brain may contribute to oxidative stress and alter localized metabolism (Joshi and Tyndale, 2006). Furthermore, long term nicotine administration is capable of causing a significant loss of white core of cerebellum (Tewari *et al*., 2010), and this probably could result in some forms of derangement in cerebellar functions.

Use of tobacco products during pregnancy has been related to some common neurobehavioral and cognitive outcomes in the offspring. Such features include, increased externalizing behaviour, decreased general cognitive function, learning and memory deficits, among others (Huizink and Mulder, 2006). The current study aimed at determining the effects of increasing doses of cigarette smoke on cerebellar cyto-architecture of adult wistar rats.

MATERIALS AND METHODS

The research work was conducted in the Animal House of the College of Health Sciences, University of Ilorin following basic ethical considerations.

Breeding of Experimental Animals

The rats were purchased from an area in Ilorin, Nigeria. They were housed in cages with adequate space to encourage free movement. They were housed under natural light and dark cycles (12hr light and 12hr dark) at room temperature and were given standard rat pellets and water *ad libitum*, and were allowed to acclimatize for two weeks.

Grouping of Experimental Animals

A total of 16 male Wistar rats with an average weight of 153.24 ± 4.12 g were used for the experiment. They were grouped into four groups, each group with four rats. However, the grouping of rats was done taking into consideration their various weights.

Exposure of Animals to Cigarette Smoke

Each animal in the treated groups was exposed to cigarette smoke (Pall Mall®) daily for 28 days. Exposure time was 6.00 pm local time, and each stick of cigarette was completely burnt within an average period of 11 minutes. Four smoking chambers were constructed for the administration. They were made of plastic containers, with a hole of about 1 cm diameter created on the lid through which each cigarette was suspended with the aid of a thread. The lids were opened intermittently to prevent suffocation. Exposure of the animals was as previously reported (Omotoso *et al*., 2013), and also stated below:

Group A: Control, exposed to fresh air;

Group B: exposed to smoke from one (1) stick of cigarette;

Group C: exposed to smoke from two (2) sticks of cigarette; and,

Group D: exposed to smoke from three (3) sticks of cigarette.

Animal Sacrifice and Tissue Collection

All the Wistar rats were sacrificed 24 hours after the last exposure by cervical dislocation. The cranium of each animal was opened up using brain forceps and the whole brain was carefully removed and weighed; the cerebellum was then excised. Tissues were then fixed in formol calcium, and processed for histological observation using routine Haematoxylin and Eosin staining techniques (Bancroft and Cook, 1984).

The weights of the animals were taken at intervals during the experiment and prior to the time of sacrifice, and the cerebellar weight was also taken.

Statistical analysis:

Data were analysed using student's t-test, and presented as Mean ± SEM, with confidence interval at 95% and a P value less than 0.05 was considered statistically significant.

RESULTS

Physical Observation

The mean weight of the animals at the commencement of the experiment was 153.24 ±4.12 g. Changes in weight of animals were observed (Table 1) which showed marked reduction in body weights in all the groups exposed to cigarette when compared to the Control animals. The animals that received the highest dose of cigarette smoke (3 sticks of cigarette) had the lowest weight difference (that is, difference between the final and initial body weights), although this least growth rate was not statistically significant ($p>0.05$) when compared to the Control. However, the weight difference in animals exposed to 1 stick (Group B) and 2 sticks (Group C) of cigarette was statistically significant ($p<0.05$).

Cerebellar weights in all the treated groups decreased. While that of Group D that had the highest dose of cigarette smoke was lowest (0.2973±0.044; $p>0.05$) compared with the Control Group A (0.5287±0.057), weight reduction in other two exposed groups (Group B: 0.3623±0.007; Group C: 0.3124±0.023) was statistically significant ($p<0.05$).

The organ-body weight ratio also reduced markedly in all exposed groups in a dose- dependent pattern, with animals exposed to smoke from 3 sticks of cigarette having the least ratio compared to the Control animals.

Histological Observation

Histological sections of the cerebelli of control rats showed apparently normal architecture with distinct cortical layers: outer molecular, inner granular cell layer, between which is the single layer of large neurones called Purkinje cells; the central medullary region was also seen, made up of white matter (Figure 1). The granular cell layer was very populated with cells, unlike the molecular layer which had large numbers of unmyelinated fibres. Animals exposed to smoke from only one stick of cigarette (Group B) revealed an apparent increase in cerebellar medulla as well as decreased thickness of the middle layer of Purkinje cells (Figures 2).

Table 1: Weights of rats and cerebelli following exposure to different doses of cigarette smoke

Group	Initial weight (g)	Final weight (g)	Weight difference (g)	Cerebellar weight (g)	C-B.Wt ratio
A	131.25 ±8.17	214.70 ±9.20	83.45	0.5287 ±0.057	0.0025
B	165.48 ±4.15	198.23 ±2.67	32.75*	0.3623 ±0.007*	0.0018
C	154.80 ±1.23	196.82 ±11.01	42.02*	0.3124 ±0.023*	0.0016
D	163.67 ±5.43	192.27 ±16.53	28.6	0.2973 ±0.044	0.0015

*P<0.05, C-B.Wt= Cerebellar-Body weight

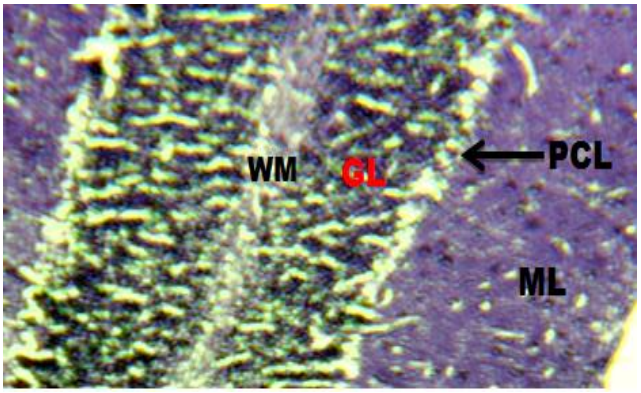

Figure 1: Photomicrograph of the cerebellum of control rat (Group A) showing apparently normal histology of highly cellular granular cell layer (GL), single Purkinje cell layer (PCL), molecular layer (ML) with fewer cells, and the central medulla of white matter (WM) (H &E x100).

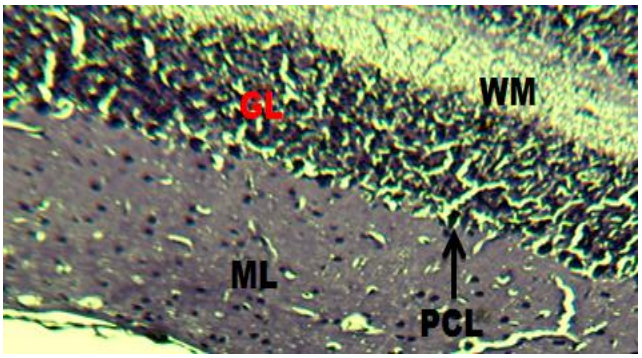

Figure 2: Photomicrograph of the cerebellum of rats exposed to one stick of cigarette (Group B) showing increased thickness of white matter (WM) compared with the Control, decreased thickness of Purkinje cell layer (PCL), with no distinctive alteration in the architecture of the granular cell layer (GL) and molecular layer (ML). (H &E x100).

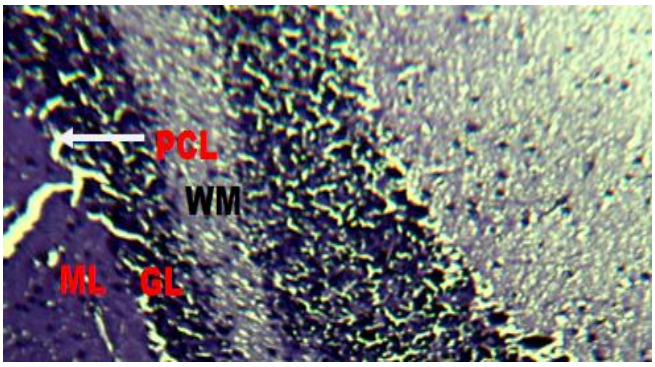

Figure 3: Photomicrograph of the cerebellum of animals exposed to two sticks of cigarette (Group C) showing a decrease in thickness of the granular cell layer (GL), Purkinje cell layer (PCL), molecular cell layer (ML) and a loss of white matter (WM) (H &E x100).

Photomicrograph of the cerebelli of animals exposed to two sticks of cigarette (Group C) showed a decrease in thickness of the cortical layers, with possible reduction in number of cells, and a loss of white matter in the medulla (Figures 3).

Animals in Group D that were exposed to the highest dose of cigarette smoke had a dose-dependent reduction in the size of the cortical layers compared with other treated groups, and a significant decrease in the number of cell bodies, and reduced white matter in the medulla (Figure 4).

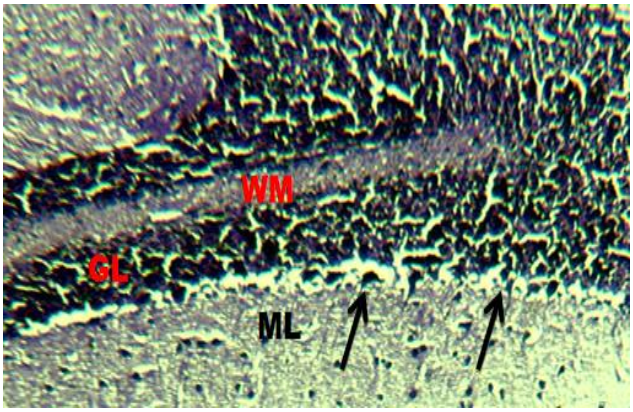

Figure 4: Photomicrograph of the cerebellum of rats exposed to three sticks of cigarette (Group D) showing further decrease in thickness of the granular cell layer (GL), decrease in the number of cell bodies in the molecular cell layer (ML), Purkinje cells (arrows), and loss of white matter (WM) (H &E x100).

DISCUSSION

Significant weight loss was observed in all the animals exposed to cigarette smoke when compared to animals in the control group, probably due to reduced food intake in the exposed groups. This was similar to previous studies on cigarette smoking which have linked weight reduction with this lifestyle both in man and experimental animals (Bishop *et al*., 2004; Bellinger *et al*., 2003; Albanes *et al*., 1987). Smoking is known to decrease insulin sensitivity (Gupta *et al*., 2006; Targher *et al*., 1997), and cause significant reduction in body weight of the animals with a significant reduction in adipose tissue (Chen *et al*., 2005), particularly white fat masses, as fat deposits might be used as an energy supply under these conditions of negative energy balance (Chen *et al*., 2005). Aside the changes in animal weight, the cerebelli of the animals in the exposed groups were also significantly reduced in weight in a dose-dependent fashion, with animals exposed to the highest dose of cigarette smoke having the lowest cerebellar weight and those exposed to the lowest dose of only one stick of cigarette having the highest cerebellar weight of the three exposed groups. In a similar pattern with the organ weights, the organ-body weight ratio dose-dependently decreased.

The development of different parts of the brain, including the cerebellum, is affected by nicotine exposure during prenatal and postnatal periods in rats (Dwyer *et al*., 2009). The adult cerebellum is also susceptible to the damaging effects of nicotine treatment by causing a significant depletion of the white core of cerebellum (Tewari *et al*., 2010). Through the process of apoptosis, interaction of nicotine receptors and nicotine results in cell death in brain tissue (Denissenko *et al*.,

1996). In the current work, both the cerebellar Purkinje cell and granular cell layers were noticed to exhibit some degree of shrinkage, or reduction in thickness, in animals exposed to cigarette smoke, and this was however more in those that received the higher doses, compared with the Control animals. Earlier studies with nicotine also revealed that administration of nicotine specifically inhibits cerebellar Purkinje cells (de la Garza *et al*., 1989), which could result in the reduction of Purkinje cells, and consequently, the middle Purkinje cell layer of the cerebellum. According to Opanashuk and colleagues (2001), activation of nicotinic acetylcholine receptors directly affect the development of primary cerebellar neuroblasts. With increasing dosage of cigarette smoke, a progressive loss of white matter was observed, which correlates with the work of Tewari *et al*. (2010) that long-term nicotine exposure during adulthood resulted in loss of white matter of the cerebellum in rat model system.

It could be concluded that increased exposure to cigarette smoke causes a progressive neurotoxicity on cerebellar cytoarchitecture, which could serve as basis for some cerebellar lesions in individuals who smoke.

REFERENCES

Albanes, D., Jones, D.Y., Micozzi, M.S., Mattson, M.E. (1987). Association between smoking and body weight in the US population: analysis of NHANES II. *Am J Public Health* 77:439–444.

Bancroft, J., Cook, H. (1984). Manual of Histological techniques. Edinburgh: Churchill Livingstone.

Bellinger, L., Cepeda-Benito, A., Wellman, P.J. (2003). Meal patterns in male rats during and after intermittent nicotine administration. *Pharmacol Biochem Behav* 74:495–504.

Bishop, C., Parker, G.C., Coscina, D.V. (2004). Systemic nicotine alters whole-body fat utilization in female rats. *Physiol Behav* 80:563–567.

Chen, H., Vlahos, R., Bozinovski, S., Jones, J., Anderson, G.P., Morris, M.J. (2005). Effect of short-term cigarette smoke exposure on body weight, appetite and brain Neuropeptide Y in mice. *Neuropsychopharmacology* 30:713-719.

de la Garza, R., Freedman, R., Hoffer, J. (1989). Nicotine-induced inhibition of cerebellar Purkinje neurons: specific actions of nicotine and selective blockade by mecamylamine. *Neuropharmacology* 28(5):495-501.

Denissenko, M. F., Pao, A., Tang, M. (1996). Preferential formation of Benzo(a)pyrene adducts at lung cancer mutational hot spots in p53. *Science* 274:430-432.

Dwyer, J.B., McQuown, S.C., Leslie, F.M. (2009). The dynamic effects of nicotine on the developing brain. *Pharmacol Ther*. 122(2):125-39.

Fechter, L.D., Annau, Z. (1977). Toxicity of mild prenatal carbon monoxide exposure. *Science* 197(4304):680-682.

Gupta, V., Tiwari, S., Agarwal, C.G., Shukla, P., Chandra, H., Sharma, P. (2006). Effect of short term cigarette smoking on insulin resistance and lipid profile in asymptomatic Adults. *Indian J Physiol Pharmacol* 50(3):285–290.

Henderson, A.J. (2008). The effects of tobacco smoke exposure on respiratory health in school-aged children. *Paediatric Respiratory Reviews* 9(1): 21-28.

Huizink, A.C., Mulder, E.J. (2006). Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neurosci Biobehav Rev*. 30(1):24-41.

Jill, S., Halterman, J.S., Fagnano, M., Conn, K.M., Szilagyi, P.G. (2006). Do parents of urban children with persistent asthma ban smoking in their homes and cars? *Ambulatory Pediatrics* 6(2):115-119.

Joshi, M., Tyndale, R.F. (2006). Regional and cellular distribution of CYP2E1 in monkey brain and its induction by chronic nicotine. *Neuropharmacology* 50(5):568-575.

Li, Y., Wang, H. (2004). In utero exposure to tobacco and alcohol modifies neurobehavioral development in mice offspring: consideration a role of oxidative stress. *Pharmacol Res.* 49(5):467-73.

Mactutus, C.F., Fechter, L. (1984). Prenatal exposure to carbon monoxide: learning and memory deficits. *Science* 223(4634):409-411.

Omotoso, G.O., Kadir, R.E., Alabi, J.T., Alabi, A.S., Oyabambi, A.O. (2013). Exposure to cigarette smoke altered the cytoarchitecture and anti-oxidant activity of the frontal cortex in Wistar rats. *International Journal of Biological and Chemical Sciences* 7(4):1595-1601.

Opanashuk, L.A., Pauly, J.R., Hauser, K.F. (2001). Effect of nicotine on cerebellar granule neuron development. *Eur J Neurosci*. 13(1):48-56.

Palozza, P., Serini, S., Trombino, S., Lauriola, L., Ranelletti, F.O., Calviello, G. (2006). Dual role of ß-carotene in combination with cigarette smoke aqueous extract on the formation of mutagenic lipid peroxidation products in lung membranes: dependence on pO_2. *Carcinogenesis* 27(12):2383-2391.

Targher, G., Alberiche, M., Zenere, M.B., Bonadonna, R.C., Muggeo, M., Bonora, E. (1997). Cigarette smoking and insulin resistance in patients with, noninsulin- dependent Diabetes Mellitus. *The Journal of Clinical Endocrinology & Metabolism* 82(11)3619–3624.

Tatum, A.J., Shapiro, G.G. (2005). The effects of outdoor air pollution and tobacco smoke on asthma. *Immunology and Allergic Clinics of North America* 25(1):15-30.

Tewari, A., Hasan, M., Sahai, A., Sharma, P.K., Rani, A., Agarwal, A.K. (2010). White core cerebellum in nicotine treated rats: a histological study. *J. Anat. Soc. India* 59(2):150-153.

Niger. J. Physiol. Sci. 29(June 2014) 047 –053
www.njps.com.ng

Niger. J. Physiol. Sci.

Bioinformatic analysis of dihydrofolate reductase predicted in the genome sequence of *Lactobacillus pentosus* KCA1

***Kingsley C. Anukam[1] and Uche Oge[2]**

[1]TWAS Genomic Research Unit, Department of Medical Laboratory Science, [2]Department of Physiology, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.

Summary: Physiologic studies of Lactobacillus species show that some species cannot synthesize folate *de novo*, which is required for growth. Folate plays a critical role in regulating the amount of tetrahydrofolate in the cell that is utilized for DNA replication, and proliferation of the erythropoietic system. We recently sequenced the genome of *Lactobacillus pentosus* KCA1, isolated from a Nigerian subject. The genome has open reading frames coding for the complete genes required for folate biosynthesis. Our previous study shows that rats fed with *L. pentosus* KCA1 led to enhancement of haematological parameters. Bioinformatic tool such as ClustalW algorithm was used to analyze dihydrofolate reductase (folA/dfrA) encoded in the genome sequence of *L. pentosus* KCA1 for comparative multiple sequence alignments. I-TASSER was used to predict the 3-D model structure of the protein and potential active binding site residues. Result show that two unique amino acid substitutions were found in KCA1_1610 sequence at position 85 with alanine (A-Ala85), while other strains have aspartic acid (D-Asp) for other *L. pentosus* and threonine (T-Thr) for *L. plantarum* strains at the same position. The result suggests that dihydrofolate reductase can be used as a distinguishing marker between *L. pentosus* KCA1 and other pentosus including *L. plantarum* strains. The secondary structure prediction with I-TASSER revealed 5 alpha helices and 8 beta-strands. Twelve binding site residues were predicted in KCA1_1610 relative to the template protein 2zzaA in protein database (PDB). The predicted structure of KCA1_1610 dihydrofolate reductase can serve as a new template as an addition to structural genomics and generation of models for use in drug screening and physiological function inference.

Keywords*: Lactobacillus pentosus*, folate biosynthesis, dihydrofolate reductase, probiotics

**Address for correspondence:* kanukam@gmail.com

Manuscript Accepted: May, 2014

INTRODUCTION

Lactobacilli are known to occur in a variety of microecology and several species colonize the human gut and vagina. Some species have been proven to confer health benefits on the host when given in adequate amounts, a concept known as probiotics (Lebeer, 2008). The mechanisms of action following these health-promoting characteristics are still investigated by several scientists. Probiotic bacteria have been shown to improve the immune system and biosynthesis of vitamins is one the suggested causal relationships of the health benefits. In many metabolic pathways of the body, folates represents an important nutritional constituent that are utilized for DNA replication, and proliferation of leucocytes, erythrocytes and enterocytes (Fuchs et al., 2002).

The microbiota of the human colon is known to produce vitamin K (menaquinones) and most of the water-soluble vitamins of group B, including biotin, nicotinic acid, folates, riboflavin, thiamine, pyridoxine, panthotenic acid, and cobalamin (Hill, 1997). In fact, the whole genetic information of the microbial community (microbiome) of the human distal gut revealed a variety of COGs (Clustered Orthologous Groups), which are involved in the synthesis of several essential vitamins (Gill et al., 2006). Unlike dietary vitamins, which are mainly absorbed in the proximal part of the small intestine, the uptake of microbial vitamins predominantly occurs in the colon (Said & Mohamed, 2006). Colonocytes appear to be able to absorb biotin, thiamin, folates, riboflavin, panthotenic acid, and menaquinones, indicating that the microbiota-produced vitamins may contribute to the systemic vitamin levels and especially to the homeostasis of the vitamins in the localized epithelial cells (Ichihashi et al., 1992). Absorption of folate occurs primarily in the duodenum and upper jejunum while the colon apparently represents a major repository of folate and the vitamin produced by the colonic microbiota

exceeds dietary intake and affects the folate status of the host. It is produced in large quantities by intestinal bacteria, mainly as monoglutamylated folate, the form that is absorbed at the highest rate (Kim et al., 2004). It has been demonstrated that the folate synthesized by intestinal bacteria can be absorbed across the colon and used by the host (Aufreiter et al., 2009).

The genome sequence of an increasing number of strains of Lactobacillus species has provided a major contribution to the knowledge of folate biosynthesis by these bacteria (De Crécy-Lagard et al., 2007). The analysis of genome sequences for predictable metabolic pathways using KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http://www.genome.jp/kegg) suggests that the ability to synthesize pABA de novo is absent among all the sequenced members of the genus Lactobacillus. In fact, the enzymes which are necessary for chorismate conversion into pABA are lacking. Moreover, the shikimate pathway for chorismate production is complete only in the strains of *L. plantarum* WCFS1 (Kleerebezem et al., 2003) and *L. pentosus* KCA1 (Anukam et al., 2013), while it is absent or partial in all the other lactobacilli (Green et al., 1996).

The genome of *Lactobacillus pentosus* KCA1 dedicates 121 genes for the biosynthesis of cofactors including twenty-four open reading frames (ORF) coding genes involved in the biosynthesis of folate (Anukam et al., 2013). Our previous study provided useful information on the immuno-regulatory potentials of *Lactobacillus pentosus* KCA1 and suggest that ingestion of the strain will not cause any deleterious effect on the haematological parameters in healthy subjects as the red cell indices of Sprague-Dawley rats were enhanced (Anukam et al., 2014).

The objectives of the present study are to use bioinformatic tools to analyze the dihydrofolate reductase (folA or dfrA) encoded in the genome sequence of Lactobacillus pentosus KCA1 and second to determine the 3-D model structure of the protein and potential active binding site residues.

MATERIALS AND METHODS

Location of folate biosynthetic genes in *L. pentosus* KCA1

Folate biosynthesis repertoire was analyzed from RAST SEED database (Aziz et al., 2008). The Ensembl genome annotation system developed jointly by the European Buoinformatic Institute (EBI) and the Wellcome Trust Sanger Institute was used for the location, extraction of the nucleotide base sequence or open reading frame (ORF) and the amino acid translation of the dihydrofolate reductase from *L. pentosus* KCA1 (KCA1_1610) (http://ensemblgenomes.org/id/EIW13833).

Sequences similar to dihydrofolate reductase of *L. pentosus* KCA1 were searched for in UniProt® database using BLASTp algorithm.

Multiple sequence alignments

The amino acid translations from the nucleotide bases of 16 bacterial organisms were selected from the BLASTp of UniProt® database (http://www.uniprot.org/) based on product annotation hit (dihydrofolate reductase), gene name, % identity, matrix score and E-value. These 16 amino acids sequences including the sequence of *L. pentosus* KCA1 (http://www.uniprot.org/uniprot/I8R625) were imported into the ClustalW algorithm for multiple sequence alignments.

Prediction of secondary structure, 3-D model, similarity structure in PDB, functional and binding sites predictions using I-TASSER.

The iterative threading assembly refinement (I-TASSER) server is a four stage integrated platform for automated protein structure and function prediction based on the sequence-to-structure-to-function paradigm (Roy *et al.*, 2010). The amino acid sequence of *L. pentosus* KCA1_1610 was submitted online (Yang, 2008) for the prediction of the 3D structure, similar structures in PDB, function and the binding site by this integrated algorithm.

RESULTS

The RAST subsystem identified all the genes involved in the pterin branch and *de novo* biosynthesis of folate in *L. pentosus* KCA1 (**Figure 1**). Three dihydrofolate reductase dfrA or FolA (KCA1_1610, KCA1_2309, KCA1_2335) genes were located in the *L. pentosus* KCA1 chromosome. Dihydrofolate reductase KCA1_1610 was found in contig AKAO01000040.1 from the DNA assembly and located between 1,801,463 and 1,801,954 along the *L. pentosus* KCA1 chromosome.

Basic Local Alignment Search Tool for proteins (BLASTp) from the UNIPROT® database yielded 250 hits with KCA1_1610 amino acid sequence. Sixteen Lactobacillus species were selected based on the same number of amino acid sequence, annotation name- dihydrofolate reductase, percentage identity (83-100 %), matrix score and e-value cutoff of $4.0\times10e^{-98}$ (**Table 1**). Two Lactobacillus species, notably, *L. pentosus* MP-10 and *L. pentosus* IG1 had 98.0 % amino acid sequence identity to KCA1_1610 with e-value 4.0×10e-115 and 2.0×10e-114 respectively. The remaining Lactobacillus species had 83.0% amino acid sequence identity.

ClustalW multiple sequence alignments showed that *L. pentosus* KCA1_1610 dihydrofolate reductase had significant amino acid sequence identity with the dihydrofolate reductase from the selected

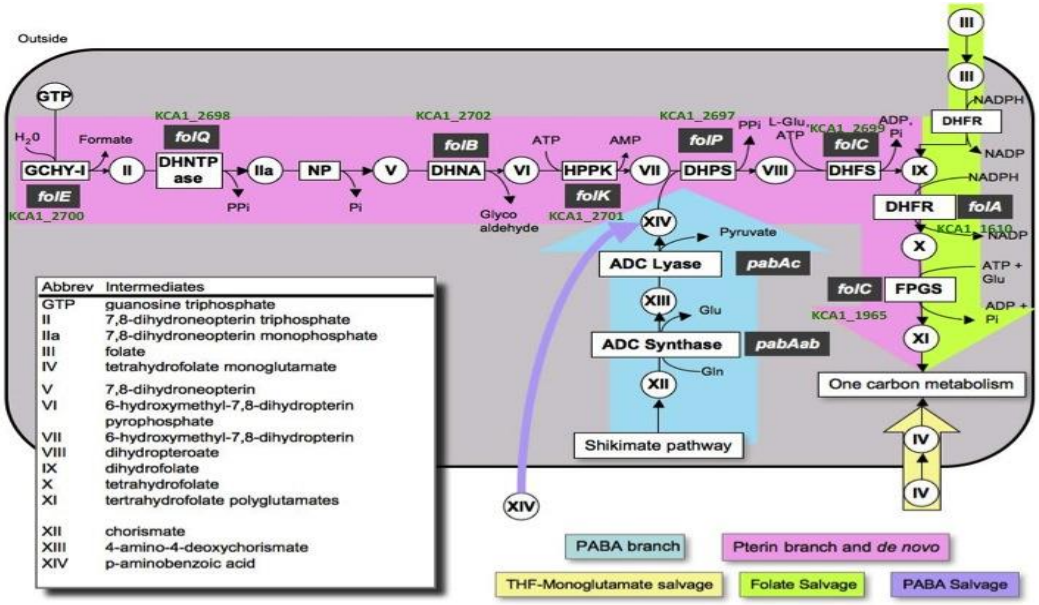

Figure 1: Folate biosynthesis pathways as identified with RAST Subsystems. Gene names are white on grey and the corresponding *L. pentosus* KCA1 genes are in green. ADC, aminodeoxychorismate; DHFR, dihydrofolate reductase; DHFS, dihydrofolate synthase; DHNA, dihydroneopterin aldolase; DHNTPase, dihydroneopterin triphosphate pyrophosphatase; DHPS, dihydropteroate synthase; FPGS, folylpolyglutamyl synthase; HPPK, hydroxymethyldihydropterin pyrophosphokinase.

```
                                                    1
F9UPJ9-L_plantarum_WCFS1                            MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
S2VAP5-L_plantarum_IPLA88                           MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
U6Z0T8-L_plantarum_WJL                              MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
R4PQX9-L_plantarum_P-8                              MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
D7V9P3-L_plantarum_ATCC_14917                       MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
M4KHV3-L_plantarum_ZJ316                            MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
U2INY3-L_plantarum_2165                             MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHIVMGRKTFAGF 50
C6VQJ3-L_plantarum_JDM1                             MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
R9X2L3-L_plantarum_16                               MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
H3P3R2-L_plantarum_NC8                              MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
M7CV21-L_plantarum_UCMA_3037                        MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
U2I5L6-L_plantarum_2025                             MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
E1TSF5-L_plantarum_ST-III                           MIALIWAEDQNGLIGNQGQLPWHLPADMQRFKALTTGHHVVMGRKTFAGF 50
F6IRR5-L_pentosus_MP-10                             MIALIWAEDQNGLIGKQGQLPWHLPADMHRFKTLTTGHHVVMGRKTFMGF 50
G0LZK9-L_pentosus_IG1                               MIALIWAEDQNGLIGKQGQLPWHLPADMHRFKTLTTGHHVVMGRKTFMGF 50
I8R625-L_pentosus_KCA1                              MIALIWAEDQNGLIGKQGQLPWHLPADMHRFKTLTTGHHVVMGRKTFMGF 50
                                                    **************:************:***:******:*******:**

F9UPJ9-L_plantarum_WCFS1                            KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
S2VAP5-L_plantarum_IPLA88                           KRPLPRRTNWVLSRQSDLKLPPEAHQLADVAAIQTLAAAHPDEPIFVIGG 100
U6Z0T8-L_plantarum_WJL                              KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
R4PQX9-L_plantarum_P-8                              KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
D7V9P3-L_plantarum_ATCC_14917                       KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
M4KHV3-L_plantarum_ZJ316                            KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
U2INY3-L_plantarum_2165                             KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
C6VQJ3-L_plantarum_JDM1                             KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
R9X2L3-L_plantarum_16                               KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
H3P3R2-L_plantarum_NC8                              KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
M7CV21-L_plantarum_UCMA_3037                        KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
U2I5L6-L_plantarum_2025                             KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
E1TSF5-L_plantarum_ST-III                           KRPLPRRTNWVLSRQSDLKLPPEVHQLADVAAIQTLAAAHPDEPIFVIGG 100
F6IRR5-L_pentosus_MP-10                             KRPLPHRTNWVLSQQSDLALPAGVQQLTDLAALKDLAAKNPDELIFVIGG 100
G0LZK9-L_pentosus_IG1                               KRPLPHRTNWVLSQQSDLALPAGVQQLTDLAALKDLAAKNPDELIFVIGG 100
I8R625-L_pentosus_KCA1                              KRPLPHRTNWVLSQQSDLALPAGVQQLTDVAALKALAAKNPDELIFVIGG 100
                                                    *****:*******:*****:**::.:**:*:**:: ***:*** ******

F9UPJ9-L_plantarum_WCFS1                            AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
S2VAP5-L_plantarum_IPLA88                           AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
U6Z0T8-L_plantarum_WJL                              AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
R4PQX9-L_plantarum_P-8                              AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
D7V9P3-L_plantarum_ATCC_14917                       AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
M4KHV3-L_plantarum_ZJ316                            AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
U2INY3-L_plantarum_2165                             AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
C6VQJ3-L_plantarum_JDM1                             AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
R9X2L3-L_plantarum_16                               AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
H3P3R2-L_plantarum_NC8                              AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
M7CV21-L_plantarum_UCMA_3037                        AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
U2I5L6-L_plantarum_2025                             AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
E1TSF5-L_plantarum_ST-III                           AVVFEAVLPVADYLYRTRINARFDGDTWMPAVDYTQWQLVSQQIGTVDEK 150
F6IRR5-L_pentosus_MP-10                             AVVFGAVLPIADYLYRTRINARFDGDTWMPAIDYSQWQLVTKQVGTVDDK 150
G0LZK9-L_pentosus_IG1                               AVVFGAVLPIADYLYRTRINARFDGDTWMPAIDYSQWQLVAKQVGTVDDK 150
I8R625-L_pentosus_KCA1                              AVVFGAVLPIADYLYRTRINARFDGDTWMPTIDYSQWQLVTKQVGTVDDK 150
                                                    ****:****:**********************.:**:*****::*:*****:*

F9UPJ9-L_plantarum_WCFS1                            NQYPYEFDDFRRR 163
S2VAP5-L_plantarum_IPLA88                           NQYPYEFDDFRRR 163
U6Z0T8-L_plantarum_WJL                              NQYPYEFDDFRRR 163
R4PQX9-L_plantarum_P-8                              NQYPYEFDDFRRR 163
D7V9P3-L_plantarum_ATCC_14917                       NQYPYEFDDFRRR 163
M4KHV3-L_plantarum_ZJ316                            NQYPYEFDDFRRR 163
U2INY3-L_plantarum_2165                             NQYPYEFDDFRRR 163
C6VQJ3-L_plantarum_JDM1                             NQYPYEFDDFRRR 163
R9X2L3-L_plantarum_16                               NQYPYEFDDFRRR 163
H3P3R2-L_plantarum_NC8                              NQYPYEFDDFRRR 163
M7CV21-L_plantarum_UCMA_3037                        NQYPYEFDDFRRR 163
U2I5L6-L_plantarum_2025                             NQYPYEFDDFRRR 163
E1TSF5-L_plantarum_ST-III                           NQYPYEFDDFRRR 163
F6IRR5-L_pentosus_MP-10                             NEYPYEFDDFRRR 163
G0LZK9-L_pentosus_IG1                               NEYPYEFDDFRRR 163
I8R625-L_pentosus_KCA1                              NEYPYEFDDFRRR 163
                                                    *:***********
```

Figure 2:ClustalW multiple alignment of the amino acid sequence of dihydrofolate reductase. Shown are the 16 dihydrofolate reductases indicating the gene entry name and the position of dihydrofolate reductase from *L. pentosus* KCA1 (I8R625). Residues in red show unique amino acid substitution relative to other strains. Green colour shows similar amino acid residues in L. pentosus strains. Overall homology is indicated under with *.

Lactobacillus strains especially *L. pentosus* MP-10 and IG1 (**Figure 2 and 3**). Two unique amino acid substitutions were noted in KCA1_1610 sequence at position 85 with alanine (A-Ala85) and valine at position Val131, while other strains have aspartic acid (D-Asp) for other *L. pentosus* and threonine (T-Thr) for *L. plantarum* strains at the 85 position.

The secondary structure prediction with I-TASSER revealed 5 alpha helices and 8 beta-strands as shown in **figure 4.** The first α-helix started at position 25 (P-Pro25) and ends at position 34 (L-Leu). It appears the shortest α-helix has only three amino acid residues and occurred between position 134 (Y-Tyr134) and 136 (Q-Gln136). The 3-D model of KCA1_1610 has a C-score of 1.68 and an estimated accuracy of 0.95±0.05 (TM-score-template modeling score) and 1.8±1.5Å (RMSD-root mean square deviation) based on the 10 templates used for

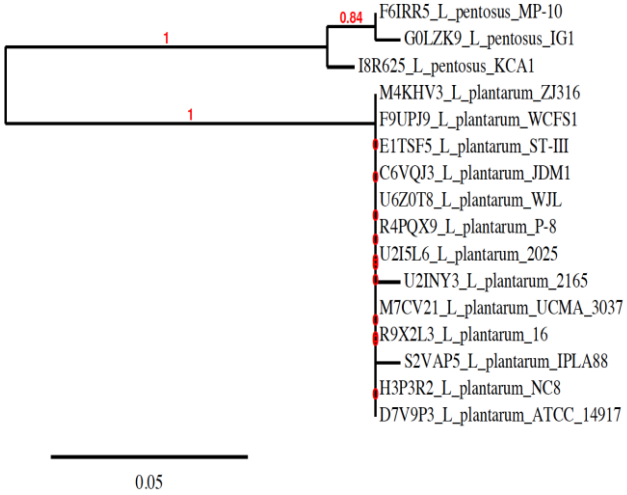

Figure 3. Dihydrofolate reductase gene tree. Confidence values for the branching order were generated by bootstrapping (based on 100 replications). The number at the nodes indicates the bootstrap values. The scale bar indicates 1 amino acid substitution per 100 amino acids

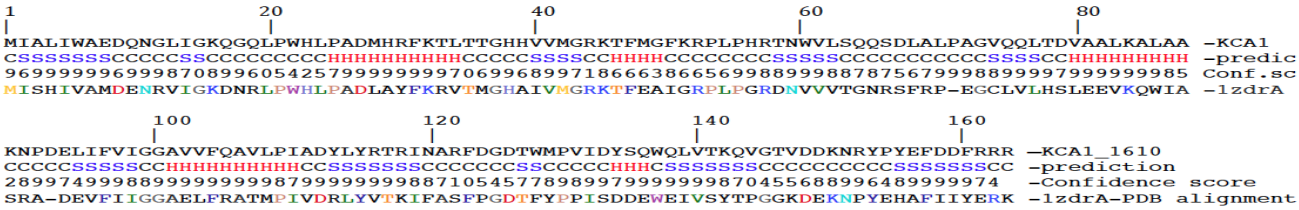

Figure 4: Predicted secondary structure with I-TASSER showing 5 alpha helices (red color) and 8 beta-strands (blue) and protein template (1zdrA) alignment with the top rank normalized Z-score

Table 1. Folate biosynthesis gene identity matrices predicted in *L. pentosus* KCA1

Gene entry ID	Organism	Length(AA)	Identity	Score	E-value	Gene name
I8R625	Lactobacillus pentosus KCA1	163	100.00%	871	4.0×10-117	dfrA KCA1_1610
F6IRR5	Lactobacillus pentosus MP-10	163	98.00%	858	4.0×10-115	LPE_00223
G0LZK9	Lactobacillus pentosus IG1	163	98.00%	853	2.0×10-114	LPENT_02812
M4KHV3	Lactobacillus plantarum ZJ316	163	83.00%	750	1.0×10-98	dfrA zj316_1848
F9UPJ9	Lactobacillus plantarum WCFS1	163	83.00%	750	1.0×10-98	dfrA lp_1869
E1TSF5	Lactobacillus plantarum (strain ST-III)	163	83.00%	750	1.0×10-98	dfrA LPST_C1499
C6VQJ3	Lactobacillus plantarum (strain JDM1)	163	83.00%	750	1.0×10-98	dfrA JDM1_1571
U6Z0T8	Lactobacillus plantarum WJL	163	83.00%	750	1.0×10-98	LPLWJ_30090
U2I5L6	Lactobacillus plantarum 2025	163	83.00%	750	1.0×10-98	N876_12100
R9X2L3	Lactobacillus plantarum 16	163	83.00%	750	1.0×10-98	Lp16_1445
R4PQX9	Lactobacillus plantarum subsp. plantarum P-8	163	83.00%	750	1.0×10-98	dfrA LBP_cg1422
M7CV21	Lactobacillus plantarum UCMA 3037	163	83.00%	750	1.0×10-98	H073_01658
H3P3R2	Lactobacillus plantarum subsp. plantarum NC8	163	83.00%	750	1.0×10-98	dfrA nc8_1602
D7V9P3	Lactobacillus plantarum subsp. plantarum ATCC 14917	163	83.00%	750	1.0×10-98	folA HMPREF0531_10866
U2INY3	Lactobacillus plantarum 2165	163	83.00%	749	1.0×10-98	N574_11250
S2VAP5	Lactobacillus plantarum IPLA88	163	83.00%	746	4.0×10-98	L103_11005

Table 2: Top 10 proteins with highly similar structural analogs in PDB (as identified by ™-align)

Rank	PDB-Hit	TM-score	RMSD[a]	IDEN[a]	Coverage
1	1zdrA	0.971	0.65	0.435	0.988
2	3ix9B	0.928	1.33	0.36	0.988
3	3e0bB	0.924	1.27	0.356	0.982
4	2w9sB	0.919	1.1	0.376	0.963
5	3tq8A	0.918	1.24	0.399	0.969
6	3f0bX	0.917	1.13	0.35	0.963
7	2zzaB	0.91	1.52	0.381	0.982
8	2d0kA	0.894	1.63	0.377	0.976
9	3jsuA	0.89	1.87	0.298	0.988
10	2blcA	0.889	1.85	0.298	0.988

(a) Ranking of proteins is based on TM-score of the structural alignment between the KCA1_1610 query structure and known structures in the PDB library. (b) RMSD[a] is the RMSD between residues that are structurally aligned by TM-align. (c) IDEN[a] is the percentage sequence identity in the structurally aligned region. (d) Coverage represents the coverage of the alignment by TM-align and is equal to the number of structurally aligned residues divided by length of the query protein.

Table 3. Top 5 enzyme homologs in PDB

Rank	$Cscore^{EC}$	PDB-Hit	TM-score	$RMSD^a$	$IDEN^a$	Cov.	EC Number	Predicted Active Site Residues
1	0.812	1zdrA	0.971	0.65	0.435	0.988	1.5.1.3	20,27
2	0.811	3fyvX	0.916	1.14	0.35	0.963	1.5.1.3	20,27
3	0.794	1ao8A	0.88	1.82	0.381	0.982	1.5.1.3	20,27
4	0.776	1ddrA	0.88	1.73	0.371	0.976	1.5.1.3	5,27,31,54,98
5	0.725	3ia4A	0.909	1.49	0.381	0.982	1.5.1.3	5,31,54,98

(a) $Cscore^{EC}$ is the confidence score for the Enzyme Classification (EC) number prediction. CscoreEC values range in between (0-1); where a higher score indicates a more reliable EC number prediction. (b) TM-score is a measure of global structural similarity between query and template protein. (c) $RMSD^a$ is the RMSD between residues that are structurally aligned by TM-align. (d) $IDEN^a$ is the percentage sequence identity in the structurally aligned region. (e) Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein.

Table 4: Template proteins with similar binding site:

Rank	$Cscore^{LB}$	PDB-Hit	TM-score	$RMSD^a$	$IDEN^a$	Cov.	BS-score	Lig. Name	Predicted binding site residues
1	0.65	2zzaA	0.911	1.51	0.381	0.982	1.55	FOL	5,6,7,20,27,28,31,32,50,54,57,98
2	0.49	2anoA	0.909	1.43	0.371	0.976	1.56	817	5,6,20,27,30,31,46,49,50,104,117
3	0.49	3kfyA	0.913	1.39	0.371	0.976	1.48	JZM	5,14,20,27,28,31,98,99,100,104,117
4	0.48	2w9sD	0.911	1.18	0.376	0.963	1.48	TOP	5,6,7,18,20,27,31,98,104
5	0.39	3tq8A	0.918	1.24	0.399	0.969	1.51	NDP	6,7,14,15,18,19,20,43,44,45,46,62,63,64,65,78,79,98,99,100,101,102,103,106,127
6	0.35	2kgkA	0.776	2.6	0.301	0.957	1.1	N22	5,50,52,55,98
7	0.26	3ia5B	0.892	1.59	0.38	0.969	1.74	PO4	43,45,46,100,103
8	0.24	1ddrB	0.887	1.65	0.371	0.976	1.35	URE	57,58,59,72,74
9	0.07	3fl9D	0.915	1.31	0.358	0.976	1.02	CA	109, 110, 111, 112, 162

(a) $Cscore^{LB}$ is the confidence score of predicted binding site. $Cscore^{LB}$ values range in between 0 and 1; where a higher score indicates a more reliable ligand-binding site prediction. (b) BS-score is a measure of local similarity (sequence & structure) between template binding site and predicted binding site in the query structure. Based on large scale benchmarking analysis, we have observed that a BS-score >1 reflects a significant local match between the predicted and template binding site. (c) TM-score is a measure of global structural similarity between query and template protein. (d) $RMSD^a$ the RMSD between residues that are structurally aligned by TM-align. (e) $IDEN^a$ is the percentage sequence identity in the structurally aligned region. (f) Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein.

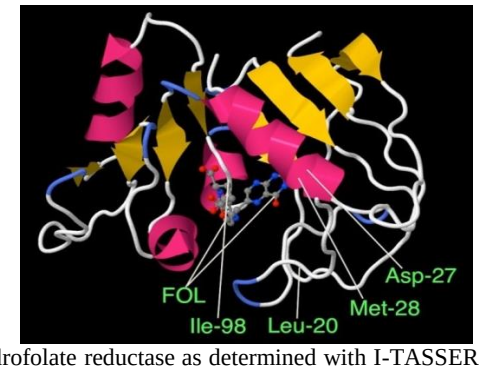

Figure 5: 3D model of KCA1_1610 dihydrofolate reductase as determined with I-TASSER based on alignments with 2zzaA-PDB. The co-ordinate file model in PDB format was visualized with Jmol molecular visualization program showing the position of the predicted binding site residues. Red color indicates the α-helices, while yellow indicates the β-pleated sheet. Magenta (+3 turns) and White (+2 turns).

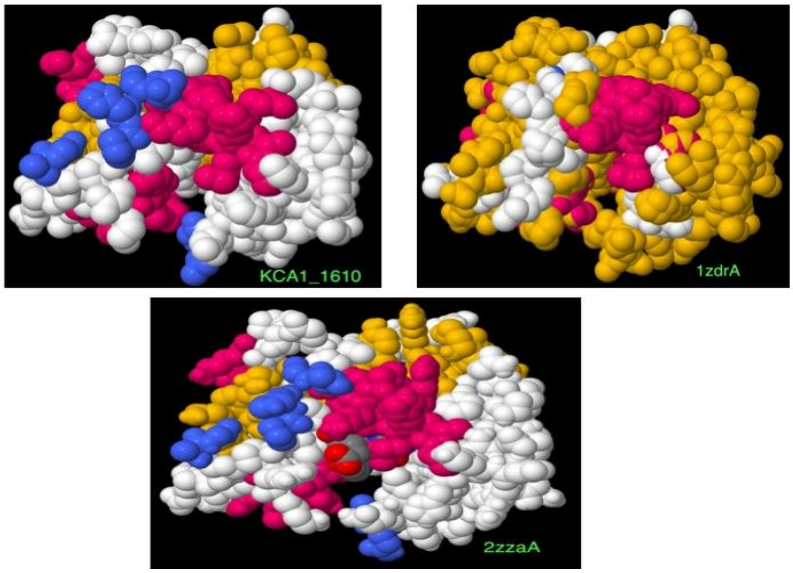

Figure 6: Comparative structure of 100% van der waal forces between KCA1_1610 dihydrofolate reductase and the top rank proteins in the PDB for similar binding site ligands (2zzaA), functional enzyme homologs and highly similar structure (1zdrA)

alignments with 1zdrA-PDB having the top rank normalized Z-score of 4.37. The co-ordinate file of the *L. pentosus* KCA1_1610 dihydrofolate reductase model was downloaded in PDB format and Jmol molecular visualization program (Hanson, 2010) was used to view the predicted structure as shown in **figure 5**. Proteins with highly similar structure in PDB as identified by TM-align are shown in **Table 2**. The protein 1zdrA PDB-hit as the top rank has a TM-score of 0.971 and a coverage of 0.988.

Five enzyme homologs were identified in PDB as having similar functions to the predicted KCA1_1610 sequence (**Table 3**). Notably, 1zdrA PDB-hit has the top rank with confidence score of 0.812 for the Enzyme Classification (EC) number (1.5.1.3). The predicted active site residues were identified as residues at position 20 and 27 in the KCA1_1610 amino acid sequence relative to three proteins in the PDB (1zdrA, 3fyvX, and 1ao8A). Predicted gene ontology (GO) terms associated with the KCA1_1610 query sequence identified 1zdrA from the PDB-hit with 6 GO terms. This protein has the top GO confidence score of 0.75 and TM-score of 0.9708.

Nine template proteins with similar binding site residues were predicted relative to KCA1_1610 sequence (**Table 4**). The template protein 2zzaA from

PDB-hit predicted binding site residues at position 5,6,7,20,27,28,31,32,50,54,57,98 relative to KCA1_1610 and has FOL (Folic Acid) as the ligand name and ligand-binding site prediction top rank with confidence score of 0.65. **Figure 6** shows the comparative structure of 100 % van der waal forces between KCA1_1610 dihydrofolate reductase and the top rank proteins in the PDB for similar binding site ligands (2zzaA), functional enzyme homologs and highly similar structure (1zdrA).

DISCUSSION

DHFR is a key enzyme in folate metabolism and play a critical role in regulating the amount of tetrahydrofolate in the cell. Tetrahydrofolate and its derivatives are essential for purine and thymidylate synthesis, which are important for cell proliferation and cell growth (Schnell, 2004). *L. pentosus* KCA1 was found to encode three genes coding for dihydrofolate reductase (EC 1.5.1.3) while *L. plantarum* WCSF1 has only one, suggesting that *L. pentosus* is equipped with more enzyme capabilities to produce folate. The amino acid composition of the gene coding for the dihydrofolate reductase with 163 amino acid residues from *L. pentosus* KCA1_1610 has a calculated molecular mass of 18, 665 daltons and it belongs to the protein family number PF00186 specific for DHFR (Myllykallio et al., 2003)

Clustal W multiple sequence alignment matrices (%) of the selected organisms clearly show that all the *L. plantarum* strains, with the exception of *L. plantarum*_2165 and *L. plantarum*_IPLA88 strains have the same sequence identity. This can be visualized from the phylogenetic gene tree (NJT) demonstrating the relatedness of the *L. plantarum* strains (**Figure 3**). The gene tree clearly shows that *L. pentosus* KCA1_1610 dihydrofolate reductase can be distinguished from other pentosus strains as the relationships at the node is apparently resolved. This suggests that KCA1_1610 can be used as a distinguishing marker between *L. pentosus* KCA1 and other pentosus including *L. plantarum* strains. However, dihydrofolate reductase of *L. pentosus* KCA1_1610 is closely related to L. *pentosus* IG1 and MP-10 than to *L. plantarum* strains. This is similar to our previous findings where the gene trees of the three conserved (housekeeping) genes (recA, dnaK, pheS) suggests that L. *pentosus* KCA1 is closer to *L. pentosus* IG1 and *L. pentosus* MP- 10 with higher percentage identity than to *L. plantarum* WCFS1 housekeeping genes (Anukam et al., 2013). The impact of the unique amino acid substitution at position 85 with alanine is yet to be determined and it would be interesting to know if there would be any difference on the biosynthesis of folate between *L. pentosus* KCA1 and *L. pentosus* MP-10 and IG1.

The secondary structure prediction was based on the alignment of KCA1_1610 to 1zdrA-PDB having top rank normalized Z-score. KCA1_1610 protein is in the same class with 1zdrA with EC number of 1.5.1.3. This class of enzyme from the physical characterization of the protein from *Bacillus stearothermophilus* indicates that it is a monomeric protein with a molecular mass of 18,694.6 Dalton, coincident with the mass of 18 694.67 Da calculated from the primary sequence (Kim et al., 2005). Determination of the X-ray structure of KCA1_1610 will provide an insight on whether the structure will turn out to be monomeric and if the calculated molecular mass of 18,665 will tally with the physical molecular mass.

Similarly, KCA1_1610 produced a hit to 1zdrA having the top rank confidence score in the enzyme homologs. C-score^{EC} is the confidence score for the Enzyme Classification (EC) number prediction. C-score^{EC} values range between 0 and 1; where a higher score indicates a more reliable EC number prediction. The predicted active binding site shows that residues Leu20 and Asp27 are found to be involved in KCA1_1610 protein, similar to three proteins (1zdrA, 3fyvX, 1ao8A) in the PDB. Recent study has shown that the nature of C-H→C transfer, and a phylogenetic analysis of DHFR sequences are consistent with evolutionary preservation of the protein dynamics to enable H-tunneling from well re-organized active sites (Francis et al., 2013).

The predicted gene ontology (GO) terms for KCA1_1610 identified 1zdrA PDB with 6 GO terms for biological functions, of which the structurally based sequence alignment of DHFRs indicates the following levels of sequence identity for KCA1_1610; 43% with 1zdrA, 37% and 35% with 3q10A and 3m08A PDB respectively. The implication of large number of GO terms associated with KCA1_1610 suggests that the protein may have biological attributes high in dihydrofolate reductase activities with a capacity to

optimize H-tunneling from donor (NADPH) to acceptor (DHF) substrates (Kim et al., 2005).

The template protein (2zzaA) with similar binding site residues was predicted to occur at 12 positions (Ile5, Trp6, Ala7, Leu20, Asp27, Met28, Phe31, Lys32, Phe50, Leu54, Arg57, Ile98) of the KCA1_1610 sequence. The template protein 2zzaA has the top rank confidence score for ligand binding site represented as FOL (Folic Acid). X-RAY DIFFRACTION with resolution of 2.00 Å shows that the template protein has three ligands including FOL (DOI:10.2210/pdb2zza/pdb).

In conclusion, bioinformatics tools have characterized the dihydrofolate reductase predicted in the genome sequence of *Lactobacillus pentosus* KCA1 as a protein with 5 alpha helices and 8 beta-strands. The protein putatively employs 12 amino acid residues as the ligand binding sites and has two unique amino acid substitutions at position ala85 and at position val131 relative to other lactobacillus species in the same clad. The structure of KCA1_1610 dihydrofolate reductase may serve as a new template that may be an addition to structural genomics and generation of models for use in drug screening and physiological function inference.

REFERENCES

Anukam, K. C., Macklaim, J. M., Gloor , G. B., Reid, G., Boekhorst , J., Renckens, B., van Hijum S. A., Siezen, R.J. (2013). Genome sequence of Lactobacillus pentosus KCA1: vaginal isolate from a healthy premenopausal woman. PLoS One. 8:e59239.

Anukam, K. C., Okungbowa, M., Mezue, C., Olise, N.A. (2014). *Lactobacillus pentosus* KCA1 does not alter haematological parameters of Sprague-Dawley albino rats, but significantly affects red cell indices pre-exposure to clinical isolate of *Escherichia coli*. Ann Biomed Sci, 13 (1): 43-52.

Aufreiter, S., Gregory, J.F., III, Pfeiffer, C.M., Fazili, Z., Kim, Y.I., Marcon, N., Kamalaporn, P., Pencharz, P.B., O'Connor, D.L. (2009). Folate is absorbed across the colon of adults: evidence from cecal infusion of (13)C-labeled (6S)-5-formyltetrahydrofolic acid. Am. J. Clin. Nutr. 90: 116–123

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes S., Glass, E. M., Kubal, M., Meyer, F., Olsen, G. J., Olson, R., Osterman, A. L., Overbeek, R. A., McNeil, L. K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G. D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics 9: 75.

De Crécy-Lagard, V., El Yacoubi, B., de la Garza, R.D., Noiriel, A., Hanson, A.D. (2007). Comparative genomics of bacterial and plant folate synthesis and salvage: predictions and validations. BMC Genomics, 8: 245.

Francis, K., Stojkovic, V., Kohen, A. (2013). Preservation of protein dynamics in dihydrofolate reductase evolution. J Biol Chem;288(50):35961-35968.

Fuchs, C.S., Willett, W.C., Colditz, G.A., Hunter, D.J., Stampfer, M.J., Speizer, F.E., Giovannucci, E.L. (2002). The influence of folate and multivitamin use on the familial risk of colon cancer in women. Cancer Epidemiol. Biomarkers Prev, 11: 227–234.

Gill, S.R., Pop, M., Deboy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B.S., Gordon, J.I., Relman, D.A., Fraser-Liggett, C.M., Nelson, K.E. (2006). Metagenomic analysis of the human distal gut microbiome. Science, 312, 1355–1359.

Green, J. C., Nichols, B.P., Matthews, R. G. (1996). Folate biosynthesis, reduction, and polyglutamylation. In Escherichia coli and Salmonella: Cellular and Molecular Biology Volume 1. Second edition. Edited by: Neidhardt FC, Curtiss R 3rd, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE. Washington DC, ASM Press :665-673.

Hill, M.J. (1997). Intestinal flora and endogenous vitamin synthesis. Eur. J. Cancer Prev, 6: S43–S45.

Ichihashi, T., Takagishi, Y., Uchida, K., Yamada, H. (1992). Colonic absorption of menaquinone-4 and menaquinone-9 in rats. J. Nutr., 122: 506–512.

Kim, T.H., Yang, J., Darling, P.B., O'Connor, D.L. (2004). A large pool of available folate exists in the large intestine of human infants and piglets. J. Nutr. 134:1389–1394.

Kim, H.S., Damo, S.M., Lee, S.Y., Wemmer ,D., Klinman, J.P. (2005). Structure and hydride transfer mechanism of a moderate thermophilic dihydrofolate reductase from Bacillus stearothermophilus and comparison to its mesophilic and hyperthermophilic homologues. Biochem. 44(34):11428-39.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O.P., Leer, R., Tarchini, R., Peters, S.A., Sandbrink, H.M., Fiers, M.W ., Stiekema, W., Lankhorst, R.M., Bron P.A., Hoffer, S.M., Groot, M.N., Kerkhoven, R., de Vries, M., Ursing, B., de Vos, W.M., Siezen R.J. (2003). Complete genome sequence of Lactobacillus plantarum WCFS1. Proc Natl Acad Sci USA, 100:1990-1995.

Lebeer, S., Vanderleyden, J., De, K., Sigrid, C. J. (2008). Genes and molecules of lactobacilli supporting probiotic action. Microbiol Mol Biol Rev, 72:728-64.

Myllykallio, H., Leduc, D., Filee, J., Liebl, U. (2003). Life without dihydrofolate reductase FolA. Trends Microbiol, 11:220-223.

Said, H.M., and Mohammed, Z.M (2006). Intestinal absorption of water-soluble vitamins: an update. Curr. Opin. Gastroenterol., 22, 140–146.

Schnell, J.R., Dyson, H.J, Wright, P.E. (2004). Structure, dynamics, and catalytic function of dihydrofolate reductase. Ann Rev Biophysics Biomolecular Structure 33 (1): 119–140.

Niger. J. Physiol. Sci. 29(June 2014) 055 –061
www.njps.com.ng

Niger. J. Physiol. Sci.

Comparative evaluation of the sperm characteristics and morphology of adult Wistar rats fed either low or normal protein-energy diets and orally dosed with aqueous *Cuscuta australis* extracts

[1]Omirinde, J.O., *[1]Ozegbe, P.C. and [2]Oyeyemi, M.O.
Departments of [1]Veterinary Anatomy and [2]Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Summary: *Cuscuta australis (C. australis*) seed and stem are commonly used as dietary supplements in a maize-meal, "Ogi", by the local population for the management of male and female reproductive dysfunctions. This study, as a part of on-going efforts, therefore, evaluated and compared the effects of Low Protein-energy (LP) and Normal Protein-energy (NP) diets on the sperm morphology and characteristics of adult Wistar rats orally dosed aqueous extracts of *C. australis* seed (LPSE and NPSE) and stem (LPST and NPST), 300mg of extract/kg body weight of rat/day, for seven days. The control groups (LPWA and NPWA) received vehicle, water. Live-dead ratio and percentage of sperms with curved tail were significantly decreased ($p<0.01$) in the NPST relative to the NPWA, LPWA, LPST, NPSE and LPSE. Total abnormal sperm counts, acephalic sperms and tailless head sperms were significantly decreased ($p<0.001$, $p<0.05$ and $p<0.001$, respectively) in the LPST and NPST relative to LPSE, NPSE, LPWA and NPWA. The LPSE, LPST and NPST showed significantly decreased ($p<0.05$) percentages of sperms with either bent mid-piece or curved mid-piece relative to the LPWA. Significantly decreased ($p<0.05$) percentage of sperms with curved mid-piece was also observed in the NPSE relative to LPWA. Protein-energy diet significantly influenced (at least $p<0.05$) the effect of each extract on sperm motility and percentage of sperms with curved tail. Stem extract significantly decreased ($p<0.01$) the percentages of acephalic sperms and tailless head sperms. Diet-stem extract interaction significantly influenced ($p<0.05$) live-dead ratio. Our data suggest that orally administered aqueous extracts of *C. australis* generally enhanced the sperm morphology and characteristics of the male Wistar rat and that the stem extract maintained sperm morphology better than the seed extract. It also showed that the stem extract decreased live-dead ratio and that the efficacy of orally administered aqueous *C. australis* stem extract may be affected by variations in dietary protein-energy levels.

Keywords*: Cuscuta australis*, protein-energy malnutrition, spermiogram, sperm morphology

**Address for correspondence:* pcozegbe@yahoo.com +234 (0) 8056010341

Manuscript Accepted: June, 2014

INTRODUCTION

Cuscuta australis R. Br, commonly called dodder, is an annual parasitic plant that forms the major flora of the tropical East and West Africa, Sudan, Madagascar, Southern Europe, Japan and Australia (Maria, 1987). Dodder is classified as a member of the Morning-Glory Family (Convolvulaceae) in older references, and as a member of the Dodder Family (Cuscutaceae) in the more recent publications (Davidson and Frey, 2005). *Cuscuta australis* has a fairly slender stem that is up to 0.5 millimetres in diameter. The flowers are about 2 millimetres long and broad (up to 3 mm. in fruit). The seeds are about 1.25 millimetres long (Verdcourt, 1963). Ye *et al*. (2002) observed that both the seeds and stems of *C. australis* contained a high amount of kaempferol, a flavonol compound.

The seed and stem of *C. australis*, locally called "Omoonigelegele", are traditionally added to a maize-meal in the South Western part of Nigeria for the management of male and female reproductive dysfunctions. Ozegbe and Omirinde (2012) reported that aqueous *C. australis* seed and stem extracts induced significant effects on the plasma concentrations of follicle stimulating hormone, luteinizing hormone, testosterone and the testicular histomorphometry of the adult male Wistar rat. However, there is dearth of literature on the effect(s) of *C. australis* extracts on the sperm characteristics and morphology.

Protein-energy malnutrition, according to Ozegbe and Omirinde (2012), induces alterations in the

morphophysiology of the seminiferous tubules of the adult Wistar rat. Decreased spermatogenesis and its' associated changes in circulating androgen and gonadotropin levels (Vawda and Mandlwana, 1990) have also been observed by Guaragna *et al*.(1986) to occur in the seminiferous tubules of malnourished rats. Malnutrition affects absorption, protein binding, distribution, biotransformation and renal elimination of xenobiotics/drugs (Krishnaswamy, 1987). However, there is no literature, to the best of our knowledge, on the effects of the interactions between protein-energy malnutrition and aqueous *C. australis* extracts on the sperm characteristics and morphology of the adult Wistar rat.

This study, therefore, evaluated the influence of protein-energy malnutrition on the characteristics and morphology of the spermatozoa of adult Wistar rats orally dosed with either aqueous *C. australis* seed or stem extracts, three possible scenarios in the developing world plagued with malnutrition and increasing incidence of male reproductive dysfunction.

MATERIALS AND METHODS

Experimental animals and their feeding

Thirty adult male Wistar rats, free from any observable ailment, obtained from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, were used for this study. The rats weighed between 200-250g at the commencement of the experiment. All the rats were kept in galvanized wire mesh cages in a fly-proofed house, under hygienic conditions, in six groups of five animals each. The two rat feed formulations of Akingbemi *et al.* (1996), Normal Protein-energy (NP, 16.55% total protein, 16.04 $mJKg^{-1}$ gross energy) and Low Protein-energy (LP, 6.21% total protein, 18.11 $mJKg^{-1}$ gross energy) diets, were used throughout the experiment. The feed rations and drinking water were supplied *ad libitum* throughout the duration of the experiment.

Experimental design

The 30 adult rats were randomly assigned to two dietary protein-energy groups; Low Protein-energy (LP) and Normal Protein-energy (NP) diets. Each dietary group, LP and NP, was further divided into three groups, each containing five rats, as follows: (i) untreated control groups that received vehicle, distilled water, only (LPWA, NPWA), (ii) treated groups that received aqueous extract of *Cuscuta* seed (LPSE, NPSE) and (iii) treated groups that received aqueous extract of *Cuscuta* stem (LPST, NPST). All rats were placed on the appropriate diet two weeks before commencement of the experiment.

Preparation of aqueous extracts and dosing

Mature seeds and stems of *C. australis*, collected from Abeokuta, Ogun State, Nigeria, were identified, numbered (UIH-22351) and deposited at the Department of Botany, University of Ibadan, Ibadan, Nigeria. One hundred and fifty grams of each sample was dried, powdered, decocted, and refluxed three times with 450mL of water, and then filtered as earlier reported by Ozegbe and Omirinde (2012). The filtrates were concentrated by rotary vacuum evaporation and then lyophilized with a freeze dryer. The yield of aqueous extracts of the seeds and stems were 15.33% and 13.33% (w/w) respectively. The lyophilized powder was dissolved in distilled water (300mg of extract in 1ml of distilled water) before oral administration to the experimental animals in accordance with the procedure of Yen *et al*. (2007).

After the 14 days of stabilization, each rat in the *Cuscuta* seed-treated (LPSE, NPSE) and the *Cuscuta* stem-treated (LPST, NPST) groups was weighed using a digital balance (Scout Pro. SPU 402, OHAUS Corporation, Pine Brook, New Jersey, USA) and each rat in each group administered by oral gavage with 300mg/kg daily of the appropriate aqueous extract for seven days. The control groups (LPWA and NPWA) were also weighed and received only distilled water (1ml/kg body weight) by oral gavage.

Semen collection

The animals were weighed, deeply anaesthetized with ketamine (100mg/kg bodyweight) and xylazine (10mg/kg bodyweight) combination, and sacrificed on Day 8 of the experiment. Semen samples were collected from the *cauda epididymidis*.

Sperm motility

The percentage of sperm cells in a unidirectional progressive movement over a field on a slide was observed, using a light microscope as described by Zemjanis (1977). Briefly, a small drop of semen was placed on a warmed slide, mixed with one drop of warm sodium citrate and covered with a glass slip. Sperm cells moving in a straightforward unidirectional motion were counted while sperm cells moving in circles, in backward direction or showing pendulating movement were excluded.

Live-Dead ratio or Percentage liveability (%)

One drop of semen was mixed with one drop of eosin-nigrosin stain on a warm slide as described by Wells and Awa (1970). A thin smear was then made from the mixture of semen and stain. The smear was then air-dried and a total of four hundred sperms observed under the microscope. The live and the dead sperm cells were separately counted and the ratio of the live to dead sperm cells was calculated according to the method of Zemjanis (1977).

Sperm morphological defects

Morphological defects in a total of 400 sperm cells were determined using the method of Wells and Awa (1970). Briefly, a drop each of Wells and Awa stain and semen were placed on a warm slide, mixed, and

with another slide, a smear was made. The stained smear was then air dried and viewed under the light microscope. The defects were classified as described by Bloom (1973) and Parkinson (2001).

Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) and two-by-two random block design ANOVA. The group means were separated by Duncan's Multiple Range Test (DMRT). The level of significance was $p \leq 0.05$. Results were presented as mean ± standard error of mean (SEM).

RESULTS

Control versus aqueous seed and stem extracts

Sperm motility

There were no significant differences ($p>0.05$) between the sperm motility of NPWA, LPSE, NPSE, LPST and the NPST. There were also no significant differences between the sperm motility of the LPWA, LPSE, NPSE and the LPST. However, sperm motility was significantly decreased ($p<0.05$) in the LPWA relative to NPWA and NPST (Fig.1)

Live dead ratio or Percentage liveability (%)

Live dead ratio of sperm cells was significantly decreased ($p<0.01$) in NPST relative to NPWA, LPWA, NPSE, LPSE and LPST (Fig. 2).

Total abnormal sperm counts as a percentage (%) of total sperm count

There were no significant differences ($p>0.01$) between the total abnormal sperm cells of NPWA and LPWA. However, the NPST and LPST showed significant reductions ($p<0.001$) in the total abnormal sperm counts relative to NPWA, LPWA, NPSE and LPSE (Fig.3).

Sperms with tailless heads (normal head without tail) as a percentage (%) total sperm defects

The NPWA, LPWA, NPSE and LPSE showed significant increases ($p<0.01$) in the percentages of tailless head spermatozoa relative to the NPST and LPST (Table 1).

Sperms with headless tails (acephalic sperms or normal tail without head sperms) as a percentage (%) of total sperm defects

There were no significant differences ($p>0.05$) between the percentages of headless tail sperms of the NPWA and NPSE, as well as between those of the LPWA and LPSE. However, percentages of headless tail sperm were significantly decreased ($p<0.05$) in both the LPST and the NPST relative to the LPWA, NPWA, LPSE and NPSE as shown in Table 1.

Sperms with curved tails (% of total sperm defects)

Percentages of sperms with curved tails were significantly decreased ($p<0.001$) in the NPST relative to NPWA, LPWA, NPSE, LPSE and LPST (Table 1). Non-significant differences ($p>0.01$) were, however, observed between the percentages of sperms with curved tails in the NPWA, LPSE and LPST as well as between those of LPWA and NPSE.

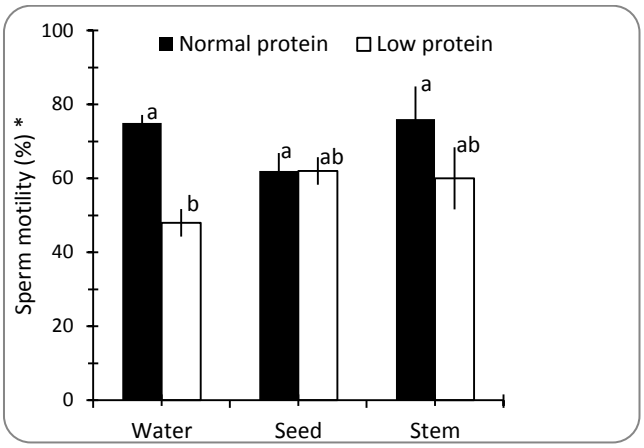

Fig.1. Mean ± SEM sperm motility (%) of *Cuscuta australis*-treated rats fed either normal or low protein-energy diets. *p < 0.05, Values with different superscripts are significantly different.

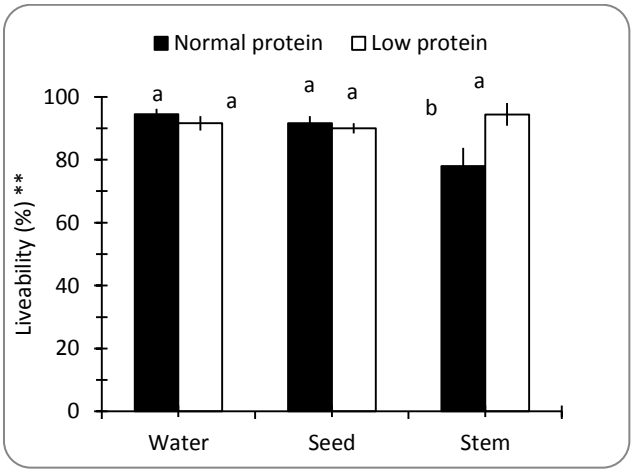

Fig.2. Mean ± SEM sperm liveability or live-dead ratio (%) of *Cuscuta australis*-treated rats fed either normal or low protein-energy diets. **p < 0.01, Values with different superscripts are significantly different

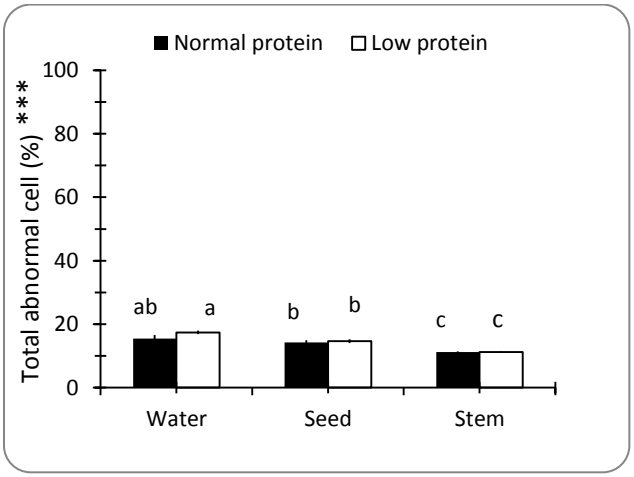

Fig.3. Mean ± SEM total abnormal sperm cells count (%) of *Cuscuta australis*-treated rats fed either normal or low protein-energy diets. ***p < 0.001, Values with different superscripts are significantly different.

Table 1: Sperm morphological defects (%) of rats that were fed either normal or low protein-energy diets and orally administered aqueous extracts of either Cuscuta australis seed or stem.

	NPWA	LPWA	NPSE	LPSE	NPST	LPST
TH**	2.24 ± 0.34^{a}	2.47 ± 0.39^{a}	1.92 ± 0.28^{a}	2.02 ± 0.27^{a}	1.07 ± 0.15^{b}	1.03 ± 0.14^{b}
HT*	1.49 ± 0.08^{abc}	1.69 ± 0.26^{ab}	1.43 ± 0.27^{abc}	1.88 ± 0.23^{a}	1.04 ± 0.93^{c}	1.12 ± 0.12^{bc}
RT	0.62 ± 0.1	0.55 ± 0.09	0.54 ± 0.12	0.64 ± 0.06	0.49 ± 0.11	0.34 ± 0.13
Bent tail	3.04 ± 0.29	2.93 ± 0.27	2.66 ± 0.19	2.48 ± 0.12	2.06 ± 0.09	3.32 ± 1.42
CT***	2.3 ± 0.14^{ab}	3.03 ± 0.12^{a}	2.37 ± 0.17^{b}	2.28 ± 0.13^{ab}	1.97 ± 0.07^{c}	2.15 ± 0.04^{ab}
LT	0.5 ± 0.14	0.50 ± 0.11	0.49 ± 0.11	0.45 ± 0.18	0.39 ± 0.12	0.34 ± 0.12
BM*	2.74 ± 0.21^{ab}	3.13 ± 0.23^{a}	2.47 ± 0.1^{ab}	2.12 ± 0.43^{b}	2.07 ± 0.06^{b}	2.0 ± 0.33^{b}
CM*	2.67 ± 0.24^{ab}	3.28 ± 0.36^{a}	2.32 ± 0.09^{b}	2.09 ± 0.45^{b}	2.17 ± 0.15^{b}	2.16 ± 0.05^{b}

Values in the same row with different superscripts are significantly different. *p< 0.05, **p< 0.01, *** p< 0.001 TH (Tailless head or normal head without tail), HT (Headless tail or normal tail without head or acephalic sperm), RT (Rudimentary tail), CT (Curved tail), LT (Looped tail), BM (Bent mid-piece), NPWA (Normal Protein-energy diet-Water), LPWA (Low Protein-energy diet-Water), NPSE (Normal Protein-energy diet-Seed), LPSE (Low Protein-energy diet-Seed), NPST (Normal Protein-energy diet-Stem), LPST (Low Protein-energy diet-Stem).

Table 2: Influence of diets and/or aqueous *Cuscuta australis* extracts on the total abnormal sperm counts and spermiogram (%) of the Wistar rat

Parameter	Diets (D)						Extracts (Ex)			D-Ex interaction	
	NP			LP							
	WA	SE	ST	WA	SE	ST	WA	SE	ST	SE	ST
Motility	75	62	76	48	62***	60***	61.5	62	68	**	ns
L:D	94.5	91.6	78	91.6	90	94.4	93.05	90.8	86.2	ns	*
TAC	15.5	14.21	11.22	17.14	14.66	11.26	16.32	14.44*	11.24***	ns	ns

ns (Not significant), *p<0.05, **p<0.01, ***p<0.001.Normal protein- energy (NP), Low protein-energy (LP), Control (WA), Seed (SE), Stem (ST), Total abnormal sperm/cell counts (TAC) and L: D (Live dead ratio).

Table 3: Influence of diets and/or aqueous *Cuscuta australis* extracts on the total abnormal sperm counts and spermiogram (%) of the Wistar rat

Parameters	Diets (D)						Extracts (Ex)			D-Ex interaction	
	NP			LP							
	WA	SE	ST	WA	SE	ST	WA	SE	ST	SE	ST
HT	2.24	1.92	1.086	2.47	2.02	1.03	2.36	1.66	1.08**	ns	Ns
TH	1.49	1.43	1.04	1.69	1.88	1.12	1.59	1.97	1.06***	ns	Ns
RT	0.62	0.54	0.49	0.55	0.64	0.34	0.59	0.59	0.42	ns	Ns
BT	3.04	2.66	2.058	2.93	2.48	3.32	2.99	2.57	2.69	ns	Ns
CT	2.3	2.37	1.97	3.03	2.28	2.15***	2.67	2.33*	2.06***	*	*
LT	0.5	0.49	0.39	0.50	0.45	0.34	0.50	0.47	0.37	ns	Ns
BM	2.74	2.47	2.07	3.13	2.12	2.00	2.94	2.3*	2.04**	ns	Ns
CM	2.67	2.32	2.17	3.28	2.09	2.16	2.98	2.2*	2.16**	ns	Ns

ns (Not significant), *p<0.05, ***p<0.001. Normal protein-energy (NP), Low protein-energy (LP), Control (WA), Seed (SE), Stem (ST), Tailless head or normal head without tail (TH), Headless tail or normal tail without head or acephalic sperm (HT), Rudimentary tail (RT), Curved tail (CT), Looped tail (LT), Bent mid-piece (BM) and Curved mid-piece (CM).

Percentages of sperm with either rudimentary tail or bent tail or looped tail (each is presented as a % of total sperm defects)

There were no significant differences (p>0.05) between the percentages of sperm with either rudimentary tail or bent tail or looped tail in the adult Wistar rats used in this study (Table 1).

Sperms with bent mid-piece (% of total sperm defects)

There were no significant differences (p>0.05) between the bent mid-piece percentages of NPWA, NPSE and LPWA (Table 1). However, the percentages of sperms with bent mid-piece decreased significantly (p<0.05) in the LPSE, LPST and NPST relative to the LPWA, NPWA and NPSE (Table 1).

Sperms with curved mid-piece (% of total sperm defects)

The NPSE, LPSE, NPST and LPST showed significantly decreased (p<0.05) percentages of sperms with curved mid-piece relative to the LPWA and NPWA (Table 1).There was a non-significant difference between the percentages of sperms with curved mid-piece in the LPWA and the NPWA (Table 1).

Control versus aqueous seed extract:

Sperm motility

Aqueous seed extract non-significantly altered (p>0.05) the percentage of sperm motility in the Wistar rat. The percentage of sperm motility was however, significantly decreased by the dietary protein-energy level (p<0.001) and influenced by the diet-seed interaction (p<0.01) as shown in Table 2. Generally, low protein-energy diet decreased sperm

motility but the seed extract ameliorated the dietary effect.

Live dead ratio / Percentage liveability (%)
Protein-energy diet, seed extract and diet-seed interaction had no significant influence ($p>0.05$) on the live dead ratio of sperm cells of the adult Wistar rat (Table 2).

Total abnormal sperm cells count
Seed extract, relative to the control, significantly decreased ($p<0.05$) the percentage of total abnormal sperm cell counts (Table 2). Protein-energy diet as well as diet-seed interaction induced non-significant effects ($p>0.05$) on the percentage of total abnormal sperm cells (Table 2).

Percentages of sperm with either headless tail (normal tail without head) or tailless head (normal head without tail) or rudimentary tail or bent tail or looped tail
The diet, the seed extract and the diet-seed interaction non-significantly altered ($p>0.05$) the percentages of sperms with either headless tail or tailless head or rudimentary tail or bent tail or looped tail in the adult Wistar rats used in this study (Table 3).

Sperms with curved tail (% of total sperm defects)
The percentages of sperms with curved tail observed in each treatment group of the adult Wistar rats were significantly altered ($p<0.05$) by protein-energy diet (increased by normal protein-energy; decreased by low protein-energy), seed extract (decreased) and diet-seed interaction (Table 3). The increased percentage of sperms with curved tail observed in the rat that received low protein-energy diet alone was ameliorated by the administration of the aqueous seed extract. Curved tail defect in the rats that received normal protein-energy diet was, however, mildly exacerbated by the administration of the aqueous seed extract.

Sperms with either bent or curved midpiece (each as a % of total sperm defects)
Seed extract significantly decreased ($p<0.05$) the percentages of sperms with bent mid-piece as well as those with curved mid-piece as shown in Table 3. Protein-energy diet alone and diet-seed interaction induced non-significant effects ($p>0.05$) on the percentages of sperms with either bent or curved mid-piece (Table 3).

Control versus aqueous stem extract:
Sperm motility
Low protein-energy diet significantly decreased ($p<0.001$) the sperm motility of the adult Wistar rats used in this study (Table 2). The effect of low protein-energy diet was antagonized by the stem extract. Aqueous stem extract and diet-stem interaction induced non-significant effects ($p>0.05$) on the percentages of sperm motility as shown in Table 2.

Live dead ratio (%)
Dietary status and stem extract treatment had no significant influence ($p>0.05$) on the live dead ratio of the sperm of adult Wistar rats used in this study (Table 2). The interaction between dietary protein-energy and aqueous stem extract significantly altered ($p<0.05$) the live dead ratio as shown in Table 2.

Total abnormal sperm counts
Total abnormal sperm count was significantly decreased ($p<0.001$) by the aqueous stem extract relative to the control (Table 2). Both the diet and diet-stem interaction induced non-significant effects ($p>0.05$) on the total abnormal sperm cell counts (Table 2).

Sperms with headless tail (% of total sperm defects)
Stem extract-treated rats showed significantly decreased ($p<0.001$) percentages of sperms with headless tail (Table 3). Dietary status and diet-stem interaction had non-significant influences ($p>0.05$) on the percentages of sperms with headless tail (Table 3).

Sperms with tailless head (% of total sperm defects)
Stem extract significantly decreased ($p<0.001$) the percentage of sperms with tailless head as shown in Table 3. Dietary status and diet-stem interaction had non-significant effects ($p>0.05$) on the percentages of sperm with tailless head (Table 3).

Sperms with either rudimentary tail or bent tail or looped tail (% of total sperm defects)
Dietary status, extract treatment and diet-extract interactions had no significant influence ($p>0.05$) on the rudimentary, bent and looped tails respectively (Table 3).

Sperms with curved tail (% of total sperm defects)
Significantly increased ($p<0.001$) percentage of sperms with curved tail was observed in the group of rats that received low protein-energy diet alone and this increase was ameliorated by the stem extract (Table 3). The interaction between dietary protein-energy and aqueous stem extract also was significant ($p<0.05$) as shown in Table 3.

Sperms with bent mid-piece (% of total sperm defects)
There was no diet and diet-stem effect ($p>0.05$) on the percentages of sperms with bent mid-piece (Table 3). Conversely, stem extract-treated adult male Wistar rats used in this study showed significantly decreased ($p<0.01$) percentage of sperms with bent mid-piece (Table 3).

DISCUSSION

Sperm morphology is an essential parameter that reflects the degree of normality and maturity of the sperm population in the ejaculate and correlates with fertility (Memon *et al.,* 1986). Defects of the head and mid-piece have been classified as primary defects of spermatogenesis (Schumacher and Moll, 2011), and arise during testicular degeneration (Bloom, 1950). Primary defects of spermatogenesis are more likely to

be associated with decreased fertility (Schumacher and Moll, 2011). The decreased percentages of total abnormal sperm counts as well as defects of the head and mid-piece of spermatozoa of the *C. australis*-treated rats observed in this study may be similar to the reported ability of *C. chinensis* seed extract to invigorate the reproductive system through its strong antioxidant properties (Peng *et* al., 1997; Qin *et al.*, 2000).

Low protein-energy diet has been reported by Ozegbe and Omoirinde (2012) to induce changes in the morphophysiology of seminiferous tubules of the adult Wistar rats. The observations of increased sperm motility in the low protein-energy diet-aqueous *C. australis* extracts-treated groups of rats indicate that protein-energy malnutrition-associated testicular dysfunction, reported by Ozegbe and Omirinde (2012) in this set of rats, rendered the adult rats sexually inactive akin to the effects of immaturity and senility that were earlier reported by Qin *et al.* (2000) to potentiate *C. chinensis*.

The significant effects of interactions between diet and aqueous extracts of *C. australis* (either seed or stem) on the percentages of spermatozoa with curved tail show that the tubular transport of spermatozoa in the *C. australis*-treated rats is directly or indirectly affected by the dietary status of the group. Heys and Gardner (1999) had earlier observed that protein-energy malnutrition elicited alterations in cellular physiology and organ function. Dietary regulation of microsomal cytochromes P450s (CYPs) has been demonstrated in monkeys, mice and rats (Rumack *et al.*, 1973; Czygan *et al.*, 1974; Adekunle *et al*., 1975). Lee *et al.* (1997) reported that such protein-energy malnutrition-induced changes in hepatic CYPs concentrations might be the cause of altered drug metabolism accompanying protein malnutrition.

Generally, fertility capacity is positively correlated to percentage liveability of the sperm cells (Oyeyemi and Okediran, 2007). The decreased live-dead ratio observed in the NPST group suggests that *Cuscuta* stem extract has an adverse effect on the fertility capacity of the sperm of recipients in the presence of normal protein-energy diet. This observed effect of the aqueous extract of the stem on sperm cell liveability in the presence of normal protein-energy diet needs further verification.

This study is part of on-going attempts to evaluate the influence of protein-energy diet on the effects of aqueous extracts of *C. australis* seed and stem on the reproductive system of the adult Wistar rat. Our work has shown that the aqueous extract of *C. australis* stem maintained sperm characteristics, except live-dead ratio, better than the seed extract. It has also shown that the efficacy of aqueous extracts of *C. australis* is influenced by the level of dietary protein-energy intake. These findings may be of value in the developing world where *C. australis* is being used in the traditional management of male reproductive dysfunction.

Acknowledgement
The excellent technical assistance of Dr. Adebayo Salaam, Department of Botany, University of Ibadan, in the identification of the *Cuscuta australis* used in this study is very gratefully acknowledged by the authors (PCO and OJO).

REFERENCES

Adekunle, A. A., Hayes, J. R. and Campbell, T. C. (1975). Interrelationship of dietary protein level, aflatoxin metabolism and hepatic microsomal epoxide hydrase activity. Life Sci. 21: 1785-1792.

Akingbemi, B. T., Aire, T. A., and Oke, B. O. (1996). Infection with T*rypanosoma brucei* potentiates the antifertility effect of gossypol, especially in the protein-malnourished male rat. Int. J. Androl. 19 (3):179-189.

Bloom, E. (1950). Fertility in male animals. J. Fertil. Steril. 1: 223-224.

Bloom, E. (1973). The ultrastructure of some characteristic sperm defects. *Nord. Vet. Med. 25*:283.

Czygan, P., Greem, H., Garro, A., Schaffner, F. and Popper, H. (1974). The effect of dietary protein deficiency on the ability of isolated hepatic microsomes to alter the mutagenicity of a primary and a secondary carcinogen. Cancer Res. 34:119-123.

Davidson, T and Frey, R. (2005) "Cuscuta." Gale Encyclopedia of Alternative Medicine. Retrieved from http://www.encyclopedia.com/doc/1G2-3435100236.html May 19, 2010

Guaragna, R., Rosa, N. B., Moraes, G. G., Perry, M. L. and Bernard, E. A. (1986): Effect of protein malnutrition on glycoprotein synthesis by testes of 20-day-old rats. *Int. J. Androl*. 9: 467-476.

Heys, S. D. and Gardner, E. (1999). Nutrients and the surgical patient: current and potential therapeutic applications to clinical practice. J. R. Coll. Surg. (Edinburgh). 44:283-293.

Krishnaswamy, K. (1987). Drug/Xenobiotic-metabolism, disposition and toxicity in malnutrition. *Defence Sci. J.* 37(2):133–142.

Lee, P. C., Struve, M. F., Bezerra, J. A. and Duncan, B. (1997). Effects of protein malnutrition on liver cytochrome P450s. *Nutrition Res*. 17: 1577-1587.

Maria L.G. (1987). Taxon Description. *Flora of Tropical West Africa* 8 (1):130.

Memon, M. A., Bretzlaff, R. N. and Ott, R. S. (1986). Comparison of semen techniques in goats. *Theriogenology.* 26: 823-827.

Oyeyemi, M.O. and Okediran, B.S. (2007). Testicular parameters and sperm morphology of chinchilla rabbit fed with different planes of soymeal. *Int. J. Morphol., 25(1)*:139-144.

Ozegbe,P.C. and Omirinde, J.O. (2012). Comparative morphophysiological evaluation of the testis of adult Wistar rats fed low protein-energy diet and dosed with aqueous extracts of *Cuscuta australis*. *Niger. J. Physiol. Sci*., 27(2):149-155.

Parkinson, T. (2001). Fertility and Infertility in Male animals.In: *Arthur's Veterinary Reproduction and Obstetrics* (Eds.: Noakes, D. E., Parkinson, T. J. and England, G. C. W.) 8th edition. Saunders Publishers, Edinburgh. Pp 695-750.

Peng, S. J., Lu, R. K, and Yu, L. H. (1997). Effects of *Semen cuscutae, Rhizoma curculiginis, Radix morindae officinalis* on human spermatozoon's motility and membrane function *in vitro*. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 17(3):145-147.

Qin, D. -N., She, B. -R., Shei, Y. and Wang, J. -H. (2000). Effect of flavonoids Cuscutae on the reproductive system in male rats. *Asian J. Androl.* 2:99-102.

Rumack, B. H., Holtzman, J. and Chase, H. P. (1973). Hepatic drug metabolism and protein malnutrition. *J. Pharmacol. Exp. Ther.* 186:441-446.

Schumacher, J. and Moll, H.D. (2011). Collection of semen. In: *A Manual of Equine Diagnostic Procedures* (Eds.: Schumacher, J. and Moll, H.D.). Teton NewMedia, Jackson, WY, USA (www.tetonnm.com/). Internet Publisher: International Veterinary Information Service, Ithaca NY (www.ivis.org). Document No. A5420.1011

Vawda, A. I and Mandlwana, J. G. (1990). The effects of dietary protein deficiency on rat testicular function. *Andrologia.* 22: 575-583.

Verdcourt, B. (1963). Taxon Description; Flora of Tropical East Africa. *East Africa Herbarium*. l: 1.

Wells, M. E. and Awa, O. A. (1970). New technique for assessing acrosomal characteristics of spermatozoa. *J. Diary Sci.* 53: 227.

Ye, M., Li, Y., Yan, Y., Liu, H., and Ji, X. (2002). Determination of flavonoids in *Semen Cuscutae* by RP-HPLC. *J. Pharm. Biomed. Anal.* 28: 621–628

Yen, F. -L., Wu, T. -H., Lin, L. -T. and Lin C. -C. (2006). Hepatoprotective and antioxidant effects of *Cuscuta chinensis* against acetaminophen-induced hepatotoxicity in rats. *J. Ethnopharmacol.* 111:123–128.

Zemjanis, R. (1977). Collection and Evaluation of Semen. In: *Diagnostic and Therapeutic Techniques in Animal Reproduction*. The Williams and Wilkins Company, Baltimore, pp: 139-180.

Zheng, H.Z., Dong, Z.H., and She, J., (1998). *Modern Study of Traditional Chinese Medicine.* 1st edition. Beijing Xue Yuan Press of the People's Republic of China, Beijing, pp. 4110- 4120.

Niger. J. Physiol. Sci. 29(June 2014) 063 –066
www.njps.com.ng

Niger. J. Physiol. Sci.

Normal Limits of Electrocardiogram and Cut-Off Values for Left Ventricular Hypertrophy in Young Adult Nigerians

[1]A. O. Ayoka, [1]O. Ogunlade*, [2]A. O. Akintomide, [1]R. O. Akomolafe, [3]O. E. Ajayi

Departments of [1]Physiological Sciences, [2]Medicine and [3]Medical Pharmacology and Therapeutics
Obafemi Awolowo University, Ile-Ife, Nigeria

Summary: This study assessed healthy young adults to determine the normal limits for electrocardiographic variables and cut-off values for left ventricular hypertrophy. It was a cross sectional descriptive study in which the participants were evaluated clinically by standard 12-lead resting electrocardiogram (ECG) at 25mm/s during quiet respiration. The heart rate, P wave duration, axis and amplitude, PR and QT intervals, QRS duration, axis and amplitude and T wave axis were assessed. Three hundred and twenty four (324) volunteers comprising of 175 males and 149 females aged 20 to 30 years (mean, 23.01±2.88years) participated in the study. The normal limits for heart rate, P wave duration, amplitude and axis in lead II, QRS duration and axis, T wave axis, PR interval, QT interval and QTc respectively were; 61-93beats per minute,0.08-0.12s,1.00-2.00mm,22.00-79.00^0,78.00-106.00ms,15.50-81.00^0, 24.25-69.00^0,0.12-0.19s, 0.32-0.40s and 0.36-0.44s. The cut-off values for Sokolow-Lyon, Cornell and Araoye criteria for assessment of left ventricular hypertrophy (LVH) were higher than those previously in use in medical practice. Gender difference exists in some cut-off values for LVH. This study defined the normal limits for electrocardiographic variables for young adult Nigerians. Racial factor should be taken into consideration in interpretation of ECG.

Keywords*:* Normal limits, Electrocardiogram, Cut-off values, Left ventricular hypertrophy, Young Adults

**Address for correspondence:* oogunlade@oauife.edu.ng Tel: +2348054106451

Manuscript Accepted: June, 2014

INTRODUCTION

Electrocardiogram (ECG) is a very important non-invasive cardiac investigation (Zipes, 2000). It had been established that age, gender and racial/ethnic differences exist with regards to ECG measurements (Rautaharju *et al*.1994, Macfarlane *et al*.1994, Vitelli *et al*.1998, Ogunlade *et al*. 2012).Therefore, age, gender and race/ethnicity should be considered in defining normal limits that will assists in accurate interpretation of ECG for a particular population. However, such reference data are not available especially for young adults. Most of the reference range data available in adults were established for middle aged population mainly of Caucasian extraction. Only very few studies exist with regards to ECG normal patterns of young adult of Yoruba population in the South-Western Nigeria (Araoye, 1984, Ogunlade *et al*.2012).This study aimed at defining the normal limits of important ECG variables and measurement for population of healthy young adults of Yoruba ethnic group. It also aimed at establishing the cut-off values for a few previously used ECG criteria for the diagnosis of left ventricular hypertrophy (LVH).

MATERIALS AND METHODS

This was a cross-sectional descriptive study. The target population was young adults between the ages of 20 and 30 years. Three hundred and twenty four (175 males and 149 females) healthy non-athletic young adults who participated in the study were recruited from Obafemi Awolowo University, Ile-Ife over a period of one year. For each candidate, weight and height measurement were carried out using a weighing scale(ZT-120 health scale).Body mass index(BMI) was estimated from the weight and height(BMI=weight/height^2) while blood pressure (BP) measurement was done using digital sphygmamometer (lumiscope) after five minutes of rest. Fasting blood glucose assessment was done using a glucometer with a drop of blood obtained from the fingertip with a sterile lancet. Inclusion criteria include; normal blood pressure (< 140/90mmHg), body mass index < 30kg/m^2, fasting blood glucose <7.0mmol/l and absence of symptomatic systemic diseases. Ethical clearance was obtained from the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals

Complex (OAUTHC), Ile-Ife (Ethical review reference no: 0005422).

The participants were educated about ECG procedure and written informed consent obtained from them. The chest and the limbs were exposed for electrode placement.With a three channel electrocardiograph (Dongjiang ECG-32A), the standard 12-lead ECG was recorded from the body surface by the attachment of 10 electrodes at the specific locations on the body according to internationally approved protocol (Kligfield *et al*. 2007).The chest leads(V1-V6) were recorded by the attachment of 6 electrodes to the precordium according to the conventional method;V1 at 4th intercostal space right sternal edge, V2 at 4th intercostal space left sternal edge, V3 at the point mid-way betweenV2 and V4, V4 at 5th intercostal space left midclavicular line, V5 at 5th intercostal space left anterior axillary line and V6 at 5th intercostal space left mid-axillary line.

The six limb leads (I, II, III, aVF, aVL and aVR) were recorded using four electrodes. An electrode was attached to the distal end of each limb according to the standard protocol for limb electrode placement. The standard 12-lead ECGs were recorded in supine position during quiet respiration at a speed of 25mm/s and calibration signal of 10mm/mV. The ECGs were printed out for detailed interpretation by a cardiologist. The QT interval was corrected for the heart rate using Bazett's formula (Bazzet, 1920).

Statistical Analysis

Values of ECG variables were presented as Mean ± SD. The lower and upper limits of normal were determined at 5th and 95th percentiles respectively. The cut-off values for the voltage LVH criteria were determined at 95th percentile. The data were analyzed with the aids of SPSS version16.0 software using descriptive statistics.

RESULTS

Three hundred and twenty four (324) young adults participated in the study. One hundred and seventy five (54.01%) were males while one hundred and forty nine (45.99%) were females. All participants were in sinus rhythm. The mean ± standard deviation (SD) for age(years), weight(kg), height(m), body mass index(kg/m^2), systolic blood pressure(mmHg) and diastolic blood pressure (mmHg) for both sexes were 23.01±2.88, 60.89 ±8.99, 1.67 ± 0.08, 21.70 ± 2.86, 119.77 ±11.37 and 72.27 ± 8.37 respectively.

The mean ± SD and normal limits of heart rate (beats per minute), P wave duration in lead II(s), P wave amplitude in lead II(mm), P wave axis (^{0}), QRS duration(ms), QRS axis(^{0}), T wave axis(^{0}), PR interval(s), QT interval(s) and QTc for the 324 young adults were 74 ± 9 (61-93), 0.09 ± 0.01(0.08-0.12), 1.28 ± 0.41(1.00-2.00), 56.99 ± 18.60 (22.00-79.00), 89.80 ± 9.48 (78.00-106.00), 54.31±19.82 (15.50-81.00), 49.74 ±13.11(24.25-69.00), 0.15 ± 0.02(0.12-0.19), 0.36 ± 0.02(0.32-0.40) and 0.40 ± 0.02 (0.36-0.44) respectively.

Table 1.Demographic characteristics and blood pressure according to gender

Parameters	**Male (n=175)**	**Female (n=149)**
Age(yrs)	23.71 ±2.91	22.18±2.54
Wt(kg)	63.02 ±8.59	58.38±8.84
Ht(m)	1.72 ±0.07	1.62±0.07
BMI(kg/ m^2)	21.30 ± 2.55	22.16±3.14
SBP(mmHg)	123.00 ±11.65	115.99±9.81
DBP(mmHg)	72.99 ±8.59	71.44±8.05

SBP-systolic blood pressure, DBP-diastolic blood pressure

Table 2. Gender-specific normal limits of heart rate, P wave, QRS complex, T wave, PR and QT intervals

ECG Variables	**Male (n=175)**	**Female (n=149)**
Heart rate(bpm)	61-88	61-94
P duration (s)	0.08-0.12	0.08-0.10
P amplitude (mm)	1.00 -2.00	1.00-2.00
P axis(^{0})	27.40-81.20	11.50-75.50
QRS duration(ms)	80.00-108.00	75.50-101.00
QRS axis(^{0})	11.00-82.20	23.00-77.00
T wave axis(^{0})	23.80-71.40	26.00-64.50
PR interval(s)	0.12-0.19	0.13-0.19
QTinterval(s)	0.32-0.39	0.36-0.40
QTc(s)	0.35-0.43	0.37-0.46

bpm- beats per minute, SBP-systolic blood pressure, DBP-diastolic blood pressure, QTc-corrected QT interval, s-second

Table 3. Normal limits for amplitude (mm) of R wave in limb and chest leads

Lead	Male (n=175)	Female (n=149)
I	3-11	3-13
II	6-19	6-19
III	2-13	1-14
aVL	0-6	0-5
aVF	4-15	3-16
V1	1-9	1-5
V2	2-15	2-12
V3	4-28	4-20
V4	10-33	8-25
V5	9-29	7-21
V6	6-20	6-18

Table 4. .Normal limits for depth (mm) of S wave in limb and chest leads

Lead	Male (n=175)	Female (n=149)
I	0-3	0-2
II	0-4	0-2
III	0-4	0-3
aVL	0-5	0-5
aVF	0-5	0-2
V1	5-23	3-17
V2	5-30	2-17
V3	0-19	0-9
V4	0-11	0-6
V5	0-6	0-3
V6	0-3	0-1

Table 5. Cut-off values for voltage left ventricular (LVH) hypertrophy in young adults.

Voltage Criteria	Description	Male	Female
Sokolow-Lyon	SV1 + RV5	>52mm	>38mm
	SV1 + RV6	>43mm	>35mm
Cornell	SV3 + RaVL	>25mm	>14mm
Araoye	SV2 + RV6	>50mm	>35mm
Ogunlade	(RI+RV5)/2	>20mm	>17mm
Gubner-Ungerleider	RI+SIII	>15mm	>16mm

The mean and normal limits of amplitude (mm) of R wave for leads I, II, III, aVL, aVF, V1,V2,V3,V4, V5 and V6 for 324 young adults were: 6.25 ± 2.60 (3-12), 11.92 ± 3.79 (3-19), 6.44 ± 3.84 (1-13), 2.06 ± 1.87 (0-5), 8.97 ± 3.67(4-16), 3.46 ± 2.19 (1-8), 7.35 ± 3.72 (2-14), 12.68 ± 5.91 (4-24), 18.66 ± 6.80(9-30), 15.63 ± 5.39(8-26) and 11.82 ± 3.97(6-18) respectively. The mean and normal limits of depth (mm) of S wave for leads I, II, III, aVL, aVF,V1,V2,V3,V4,V5 and V6 for the 324 young adults were: 0.48 ± 0.86(0-2), 0.82 ±1.23(0-3), 0.98 ±1.53(0-4), 1.31±1.78(0-5), 0.98 ± 1.71(0-4), 11.05 ± 5.91(3-22), 12.53 ± 7.99(2-28), 6.59 ± 5.71(0-18), 3.30 ± 3.26 (0-10), 1.39 ± 1.83 (0-5) and 0.73 ± 1.57(0-2) respectively. Table 1 showed the demographic characteristics and blood pressure of participants according to gender. Tables 2-4 showed gender specific normal limits for various ECG variables such as heart rate, P wave, PR interval, QRS axis, R wave amplitude, depth of S wave and QT interval. Table 5 showed the cut-off values for Sokolow-Lyon, Cornell, Ogunlade and Gubner-Ungerleider voltage criteria as determined at the 95th percentile of the ECG measurements for the age group.

DISCUSSION

This study provides a comprehensive description of normal limits for the ECG variables of young adults in a Negro population. This is important because data are sparse with regards to the characteristics and normal limits of ECG variable in this population. Moreover, most of the standards for interpretation of ECG utilized in Nigeria are derived from data/criteria obtained from White population such as Minnesota code (Blackburn, 1969).

This study showed that the upper normal limit for heart rate was 88 beats per minute in male and 94 beats per minutes in female. The upper limits for the heart rate for both genders were lower than the universally defined upper limit of 100beats per minute (Spodick , *et al.* 1992).Similar findings of upper limit below 100beats per minute (95 beats per minute) even at 98th percentile had been described in a Chinese population (Wu *et al* . 2003). In a study of apparently healthy young adults in the age group 20-39 years (92 males, 89 females) in the South-Western Nigeria in 1984, Araoye reported mean value of 68 ±13 beats per minutes and 79 ±11 beats per minutes for males and females respectively. By these, the upper limits for heart rate for young adults population studied were lower than 100 beats per minute.

The upper limits for P wave duration and amplitude are used as criteria for evaluation of left atrial and right atrial abnormalities respectively. Left atrial abnormality is assessed when the P wave duration in lead II is greater than 0.12s and right atrial abnormality is assessed when the P wave amplitude in lead II is greater than 2.5mm for both males and females (Mirvis and Goldberger, 2008). The upper limits for P wave duration was 0.12s in male and 0.10s in female while the upper limits for P wave amplitudes were 2mm in both sexes. These suggested that upper limits for P wave duration and amplitude need adjustment for the study population.

The normal limits for PR intervals in males (0.12-0.19s) and females (0.13-0.19s) in this study were comparable to that of the Caucasians (Mirvis and Goldberger, 2008).Similar findings were also described in a Chinese population (Wu *et al*.2003).

The upper limits for corrected QT intervals (QTc) were higher in females than males but the result was consistent with findings in other races. The upper limits for QRS duration were higher in males. However, the upper limits of QRS duration in this study were lower than the reference values defined for broad QRS complex (>120ms) for Caucasian population (Mirvis and Goldberger, 2008).This established that the QRS duration is narrower in the young adult population.

The upper limits of amplitude of R waves and depth of S waves across the limb and chest leads for the age group in the study were higher than previously recorded values. The commonly used diagnostic criteria for left ventricular hypertrophy were based on measurements of QRS voltages. Most of the criteria for the prediction of left ventricular hypertrophy such as Sokolow-Lyon criteria, Cornell criteria, Araoye code system and Ogunlade criterion were derived from the addition of two or more of the upper limits of R wave amplitude and or S wave depth in limb and or chest leads (Sokolow and Lyon 1949, Casale *et al.*1985, Araoye 1996, Hancock *et al.*, 2009, Ogunlade 2010). A few others were derived from the summation of points of some selected criteria and the outcome were given cut-off values (Romhilt and Estes,1968).These criteria were derived from studies conducted among individuals who were mostly greater than 35years in age. The standards for the 16 to 35year age group are not well established

(Hancock *et al*.2009). This study reassessed the existing criteria with a view to defining cut-off values for the most commonly used voltage criteria. It was discovered that for most of the voltage criteria assessed, the upper limits for cut-off values were higher than those previously established for middle age population (table 5).

In conclusion, gender difference exists in some cut-off values for LVH. This study defined the normal limits for electrocardiographic variables for young adult Nigerians. Therefore, among adult population, age, gender and racial factor should be taken into consideration in interpretation of ECG.

Acknowledgement
We acknowledged the contributions of members of staff of Department of Physiological Sciences, Obafemi Awolowo University, Ile-Ife to the project development.

REFERENCES

Araoye, M.A.(1984).The 12-lead electrocardiogram (ECG) healthy adult Nigerians; an investigation of a Yoruba group. A thesis submitted to College of Medicine, University of Lagos, Nigeria:1-248

Araoye, M.A.(1996). Left ventricular hypertrophy by electrocardiography: A code system applicable to Negroes. Nigerian Postgraduate Medical Journal. 3: 92-97.

Bazzet, H.C. (1920). An analysis of the time-relations of electrocardiogram. *Heart*.7: 353-70.

Blackburn, H. (1969). Classification of the electrocardiogram for population studies: Minnesota code. Journal of Electrocardiology. 2, 305-310

Casale, P.N., Devereux, R.B., Kligfied, P.,Eisenberg,R.R., Miller,D.H.,Chaudhary,B.S and M.C.Phillips.(1985).Electrocardiographic detection of the left ventricular hypertrophy: development and prospective validation of improved criteria. *Journal of American College of Cardiology*.6:572-580.

Hancock, E.W., Deal, B. J., Mirvis, D. M., Okin, P., Kligfield, P and Gettes, L.S.(2009). AHA/ACCF/HRS Recommendations for the standardization and interpretation of the electrocardiogram: Part V: Electrocardiographic changes associated with cardiac chamber hypertrophy: A scientific statement from the American Heart Association Electrocardiography and arrhythmias committee, council on clinical cardiology; the American college of cardiology foundation; and the Heart Rhythm Society: endorsed by the International Society of Computerized electrocardiology.Circulation.119: e251-e261.

Kligfield, P., Gettes, L. S., Bailey, J.J., Childers, R., Deal, B.J., Hancock, W., van Herpen, G., Kors, J. A., Macfarlane, P., Mirvis, D.M., Pahlm, O., Rautaharju, P and Wagner,G.S. (2007). Recommendations for the Standardization and Interpretation of the Electrocardiogram. Part I: The Electrocardiogram and Its Technology. A Scientific Statement From the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society. Circulation.115, 1306-1324.

Macfarlane, P.M., Mclaughlin, S.C., Devine, B., andYang, T.F.(1994). Effects of age, sex, and race on ECG interval measurements. *Journal of Electrocardiology*. 27:14-19.

Mirvis, D. M and Goldberger, A.L. (2008). Electrocardiography. In: Libby, P.,Bonow, R.O.,Mann, D. L., Zipes, D.P. and Braunwald, E.(eds). Braunwald's heart disease: a textbook of cardiovascular medicine. 8th Ed. Saunders Elsevier:149-193.

Ogunlade,O.,(2010). Prediction of LVH from average of R-wave amplitude in leads I and V5 among adult Nigerians with hypertension. *Nigerian Journal of Postgraduate Medicine*.3(1):5-14

Ogunlade,O.,Ayoka,A.O.,Akintomide,A.O.,Ajayi,O.E., Ukponmwan,O. E and Ogunlade, O.B. (2012). Gender differences in electrocardiogram of young adults in South-Western Nigeria. *European Journal of Scientific Research*.81(1):26-32.

Ogunlade,O., Ukponmwan, O.E., Ayoka, A.O., Akintomide, A.O. and Ogunlade, O.B., (2012). Standard 12-lead electrocardiogram in healthy young adults in South-Western Nigeria. *Acta Cardiologica*. 67(6):665-673.

Rautaharju, P.M., Zhou,S.H., and Calhoun H.P.(1994).Ethnic differences in ECG amplitudes in North American white,black, and Hispanic men and women. *Journal of Electrocardiology*.27: 20-31.

Romhilt, D.W and J.R. Estes. (1968). A point score system for the diagnosis of left ventricular hypertrophy.*American Heart Journal*.75:752-758.

Sokolow, M and T.P. Lyon.(1949).The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial leads. *American Heart Journal*.37:161-186.

Spodick, D.H., Raju R., Bishop, R.L.,Rifkin, R.D.(1992).Operational definition of normal sinus heart rate. *American Journal of Cardiology*.69:1245-1246.

Wu, J.,Kors,J.A., Rijnbeek ,P.R., Herpen,G., Lu, Z., and Xu,C.(2003).Normal limits of the electro-cardiogram in Chinese subjects. *International Journal of Cardiology*.87: 37-51.

Vitelli, L.L., Crow, R.S., Shahar, E., Hutchinson, R.G., Rautaharju, P.M., and Folsom, A.R.(1998). Electrocardiographic findings in a healthy biracial population. *American Journal of Cardiology*, 81:453-459.

Zipes, D.P (2000). Clinical application of electrocar-diogram. *Journal of American College of Cardiology*.36 (6)1746-1748.

Niger. J. Physiol. Sci. 29(June 2014) 071 –079
www.njps.com.ng

Niger. J. Physiol. Sci.

Glucose utilization and anti-oxidative mechanisms of the aqueous *hunteria umbellata* seed extract in alloxan-induced diabetic rats

[1*]Adeneye A.A., [2]Adenekan S.O., [3]Adeyemi O.O., [3]Agbaje E.O.

[1]Department of Pharmacology, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Lagos, Nigeria,[2]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria,[3]Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria

Summary: In South-west Nigeria, water decoctions of *Hunteria umbellata* seeds are highly valued by traditional healers in the local management of diabetes mellitus, obesity and hyperlipidemia. Previous studies hypothesized one of the antihyperglycemic mechanisms of the aqueous seed extract of *Hunteria umbellata* (*HU*) to be mediated probably via increased peripheral glucose utilization. The present study, therefore, was designed at evaluating the peripheral glucose utilization and anti-oxidative mechanisms of 50 mg/kg, 100 mg/kg and 200 mg/kg of *HU* in alloxan-induced diabetic rats in Groups IV-VI rats as well as in the control groups (Groups I-III). Experimental type 1 DM was induced in male Wistar rats through intraperitoneal injection of 150 mg/kg of alloxan monohydrate in cold 0.9% normal saline after which the diabetic rats were orally treated with 50-200 mg/kg of *HU* for 14 days. Effects of *HU* on the rat body weight, percentage body weight changes and fasting blood glucose (FBG) were determined on days 1 and 15 of the experiment. Also, on day 15 of the experiment, *HU* effect on serum insulin, liver enzyme markers, proteins, albumin, triglyceride, total cholesterol and lactate dehydrogenase as well as on hepatic tissue oxidative stress markers, liver glycogen and glucose-6-phosphatase were determined after sacrificing the rats under diethyl ether anesthesia. Results showed that oral treatments with 50-200 mg/kg of *HU* caused significant ($p<0.0001$) improvements in the weight loss caused by alloxan-induced diabetes, while causing significant ($p<0.05$, $p<0.001$ and $p<0.0001$) dose-related reductions in the FBG levels despite causing non-significant ($p>0.05$) alterations in the serum INS levels in the treated rats. Also, repeated oral treatment with *HU* caused significant ($p<0.0001$) reversal in the decrease and increase in the hepatic glycogen levels and glucose-6-phosphatase activity, respectively, caused by alloxan-induced diabetes. Similar significant ($p<0.0001$) and complete reversal effects were recorded in the serum hepatic enzyme markers, total protein, albumin, triglyceride, total cholesterol and lactate dehydrogenase as well as on hepatic tissue oxidative stress markers such as superoxidase dismutase (SOD), catalase (CAT), malonialdehyde (MDA) and reduced glutathione (GSH) of *HU*-treated rats when compared to that of untreated alloxan-induced diabetic rats. In conclusion, results of this study showed *HU* treatment to significantly ameliorate the hyperglycemia and oxidative stress in alloxan-induced diabetic rats which was mediated via increased hepatic glycogen deposit, decreased hepatic glucose-6-phosphatase activity and improvement in antioxidant/free radicals scavenging activities.

Keywords*:* *Hunteria umbellata*, Alloxan-induced diabetes, Fasting blood glucose, Liver glycogen, Glucose-6-phosphatase, Oxidative stress markers

**Address for correspondence:* adejuwon.adeneye@lasucom.edu.ng, adeneye2001@yahoo.com, +234-802-069-0946

Manuscript Accepted: June, 2014

INTRODUCTION

Diabetes mellitus (DM) is a state of carbohydrate, protein and lipid metabolic disequilibrium characterized by sustained hyperglycemia and other metabolic derangements, and resulting from pancreatic insulin insufficiency and/or due to defects in tissue insulin receptors (Frier and Fisher, 2010). Recent World Health Organization (WHO) data suggests that the current world's population affected by DM stands at 171 million and this figure is estimated to reach 366 million by the year 2030 (Wild *et al.*, 2004) with the estimated global cost of $1 trillion incurred yearly for treating it and its associated complications (Rahul *et al.*, 2006). Basically, DM is classified into two major common types, namely: insulin-dependent (type 1) DM and non-insulin dependent (type 2) DM (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002). Other forms of DM include pregnancy-related (gestational) DM, maturity-onset diabetes of the young (MODY), surgical diabetes,

drug-induced DM, *etc*. (Frier and Fisher, 2010). In all of these DM types, chronic hyperglycemia and oxidative stress have been identified as common features in both their etiopathology and pathophysiology (Robertson, 2004; Kaneto *et al.*, 2007).

Oxidative stress has been implicated in the etiology of DM since reactive oxygen species (ROS) radicals generated have been established to play a pivotal role in the development of DM complications such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular disease (Kaneto *et al.*, 2007). Oxidative stress promotes the onset and development of DM either by directly decreasing insulin sensitivity and/or causing direct cytotoxicity to the pancreatic insulin-producing β-cells (Maiese *et al.*, 2007). Documented ROS-induced cytotoxic mechanisms include lipid peroxidation as well as disruption of mitochondrial respiratory machinery (mitochondrial electron transport) which is regulated by NADPH ubiquinone oxidoreductase and ubiquinone-cytochrome *c* reductase systems (Maiese *et al.*, 2007). Thus, disruptions of mitochondrial *oxidoreductase* and *citrate synthase* activities result in significant reductions in mitochondrial oxidative and phosphorylation activities as well as reduction of the levels of mitochondrial proteins and mitochondrial DNA in adipocytes, particularly in type 2 DM (Petersen *et al.*, 2003). Oxidative stress has also been shown to trigger the opening of the mitochondrial membrane permeability transition pore which results in a significant depletion of mitochondrial NAD^+ stores and subsequently apoptotic cell injury (Maiese *et al.*, 2007). In the pancreatic tissues, these cellular events result in depletion of the β-cells population and insulin deficiency while in the skeletal muscle, it manifests as insulin resistance (Robertson *et al.*, 2003).

Hunteria umbellata (K. Schum.) Hallier f., belonging to Apocynaceae family, is a tropical rainforest tree which is locally known as "Abeere" among the Yorubas and the Binis tribes in Nigeria while in French it is known as "Demouain" (Adeneye and Adeyemi, 2009a). Ethnomedical uses of the plants include treatment of yaws and sexually transmitted infections, stomach ache and ulcers, diabetes mellitus and dysmenorrhoea (Falodun *et al.*, 2006). Previous studies have reported the antihyperglycemic (Adeneye and Adeyemi, 2009a; Adeneye and Adeyemi, 2009b; Igbe *et al.*, 2009) anti-obesity and antihyperlipidemic (Adeneye *et al*., 2010) effects of the crude aqueous seed extract of *Hunteria umbellata* (*HU*) in rats. Recent studies have reported the isolation of a new bisindole alkaloid, erinidine, from the crude alkaloid extract of *HU* (Adeneye *et al.*, 2012) which mediated an *in vivo* antihyperglycemic activity via intestinal glucose uptake inhibition (Adeneye *et al.*, 2013). Also, we have reported the *in vivo* anti-inflammatory and *in vitro* anti-oxidant activities of *HU* and its fractions (Adeneye *et al.*, 2011). In a previous study, HU was hypothesized to regulate glucose homeostasis via enhanced peripheral glucose utilization in experimental models of DM (Adeneye and Adeyemi, 2009a; Adeneye and Adeyemi, 2009b). Unfortunately, till date there has not been any study further investigating the exact mechanism by which *HU* regulates glucose through enhanced peripheral glucose metabolism and its possible anti-oxidative role in *in vivo* hyperglycemic models. In view of the above, the present study is designed at investigating the exact peripheral glucose utilization mechanism and the possible role of *HU* in attenuating the oxidative stress in alloxan-induced hyperglycemic rats (being a prototype of type 1 DM animal model). In doing this, 50-200 mg/kg/day of *HU* were administered to alloxan-induced diabetic rats for 14 days after which its antidiabetic and anti-oxidative profile and mechanisms were investigated in the treated diabetic rats.

MATERIALS AND METHODS

Plant Materials

Fresh matured fruits of *Hunteria umbellata* were collected from the deciduous forests of Imoroko Village, Atan-Ijebu in Ijebu East Local Government Area of Ogun State, in the month of December, 2012 and plant authentication was done as previously reported by Adeneye and Adeyemi (2009a). Fresh seeds from the fruits were separated and rinsed in tap water after which it was continuously and completely dried in an aerated oven preset at the temperature of 35 ºC and protected from direct sunlight for 4 week.

Preparation of the cold aqueous seed extract of Hunteria umbellata

Aqueous extract of *Hunteria umbellata* seed was prepared by soaking 50 g of powdered seed in 500 ml of distilled water and kept in the refrigerator for 72 hours. After 72 hours, the solution was continuously stirred using a magnetic stirrer for 2 hours after which the solution was filtered using a piece of clean white cotton cloth. The filtrate was then completely dried-off using an aerated oven preset at 40 ºC until a solid residue of constant weight was obtained.

Experimental Animals

Young adult male Wistar rats weighing between 110-130 g were procured from Bayo Animal Farm, Sango-Otta, Ogun State, Nigeria, after institutional ethical approval obtained. The rats were acclimatized and maintained on standard rat chow, potable drinking water and standard laboratory conditions (temperature: 28-30 ºC and humidity: 55-65%,) under natural 12 hour daylight/ night periodicity for 14 days before being used for the experiment. All rats were processed under same sham-handling using standard principles guiding the care and use of laboratory

animals as contained in the NIH publication No. 85-23 (1985).

Induction of experimental diabetes mellitus

Experimental type 1 diabetes was induced in rats using the method described by Venugopal *et al.* (1998) and as modified by Iwalewa *et al.* (2008). Rats were injected with freshly prepared 150 mg/kg body weight of alloxan monohydrates dissolved in sterile cold normal saline, given via the intraperitoneal route. The rats were then kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia which is often associated from alloxan-related hyperinsulinemia (Gupta *et al.*, 1984). Fasting blood glucose levels in rats were measured on the 3rd and 5th day post-alloxan injection and treated rats with fasting blood glucose levels equal to or above 250 mg/dl were considered diabetic and used for study.

Body weight measurement

Body weights of all rats were measured on the 1st and 15th day of the experiment, respectively, using digital mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 1st and 15th day in reference to the initial weight per group was calculated.

Experimental design and oral treatment of alloxan-induced hyperglycemic rats

Oral treatments of alloxan-induced hyperglycemic rats for 14 days are as follows:

Group I: normal control rats received 10 ml/kg and 1 ml/kg of distilled water via the oral and intraperitoneal routes, respectively, intraperitoneal alloxan, having being injected to other groups of rats

Group II: Alloxan-induced diabetic rats received 10 ml/kg of distilled water

Group III: Alloxan-induced diabetic rats orally received 5 mg/kg of glibenclamide in distilled water

Group IV: Alloxan-hyperglycemic rats orally treated with 50 mg/kg of *HU* in distilled water

Group V: Alloxan-hyperglycemic rats orally treated with 100 mg/kg of *HU* in distilled water

Group VI: Alloxan-hyperglycemic rats orally treated with 200 mg/kg of *HU* in distilled water.

Blood collection

On day 15, after an overnight fast, the final fasting blood glucose was determined before the treated rats were sacrificed after light diethyl anesthesia. After anesthesia, blood samples were collected directly from the heart chamber into 10 ml plain bottles. The blood samples obtained were immediately frozen at -70 ºC and centrifuged at 3000 rpm for 20 min to separate out the serum that was then analyzed for the biochemical assays such as serum insulin, liver enzyme markers, proteins, albumin, triglyceride, total cholesterol and lactate dehydrogenase.

Blood glucose measurement

Whole blood fasting blood glucose (FBG) of treated rats was collected by tail tipping method and determined by the glucose oxidase method of Trinder (1969) using a One Touch Basic Blood Glucose Monitoring System® (Life Scan Inc., Milpitas, California, U.S.A.). The blood glucose monitor was calibrated and validated at the beginning of, midway into and at the end of the experiment.

Measurement of serum insulin levels

Serum insulin was assayed by the modified method of Herbert *et al.* (1965) using insulin radioimmunoassay kits (BARC, Mumbai, India). Dextran coated charcoal in 0.2 M glycine buffer was used to separate bound and free insulin.

Measurement of serum liver enzyme markers, proteins, albumin, triglyceride, total cholesterol and lactate dehydrogenase

Serum activities of aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Moss and Henderson (1999). Serum total protein (TP) and albumin (ALB) levels were estimated using the methods of Kingsley and Frankel (1939), and Doumas *et al.* (1971) while that of TG and TC were assayed using standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.) on Automated Clinical System (Sychron Clinical System®, model: CX5 PRO) (Beckman Coulter Inc., Galway, Ireland). Serum lactic dehydrogenase activity (LDH) was measured by the method of Wroblewski and LaDue (1955).

Collection of liver tissues and determination of liver tissue SOD, CAT, MDA and GSH

Immediately the blood samples were collected, the liver was identified and removed and briskly rinsed in ice-cold 1.14% KCl solution in order to preserve activities of the oxidative stress markers before it was homogenized in 0.1 M tris-HCl buffer of pH 7.4 to give a 10% homogenate. This homogenate was used for the appropriate oxidative stress markers estimation. Superoxide dismutase (SOD) activity in the liver tissue was determined by the method of Kakkar *et al*. (1984) while that of liver MDA, catalase (CAT) and reduced glutathione (GSH) were determined by the methods of Kumar *et al*. (2010), Sinha (Sinha, 1972) and Kaur *et al*. (2006), respectively.

Determination of liver glycogen and glucose utilization

Liver glycogen content was measured by methods of Carroll *et al*. (1956) and Chattopadhyay *et al*. (1992), while the hepatic glucose-6-phosphatase concentration (being the rate limiting enzyme for glucose release from glycogen storage into the blood) was quantified by the method of Baginsky *et al*. (1992).

Statistical analysis

The values for the average body weights and percentage were expressed as mean ± S.D. while the biochemical values were expressed as mean ± standard error of mean (SEM) of six rats for each treatment group. Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test on GraphPad Prism (version 5.00, 2007; La Jolla, California, U.S.A.) statistical software. Significant values were considered at $p<0.05$, $p<0.001$ and $p<0.0001$.

RESULTS

Aqueous extraction of pulverized Hunteria umbellata seed

Extraction of pulverized *Hunteria umbellata* seed with distilled water yielded a deep brown, sweet-smelling solid residue weighing 7 g and giving a yield of 14%.

Effect of 50-200 mg/kg of HU on body weight in alloxan-induced diabetic rats

Table 1 shows the effect of repeated daily oral treatment with 50-200 mg/kg of *HU* on the average body weight and %weight change of treated alloxanized rats. There was a significant ($p<0.0001$) weight loss in the untreated alloxanized diabetic (Group II) rats during the treatment period when compared to that of untreated normal (Group I) rats (Table 1). However, oral treatments of the alloxan-induced diabetic rats with 50, 100 and 200 mg/kg of *HU* significantly ($p<0.0001$) improved body weight in Groups IV-V rats relative to that of untreated alloxanized diabetic (Group II) rats although these improvements were significantly ($p<0.05$) lower than that caused by glibenclamide treatment (Table 1).

Effect of 50-200 mg/kg of HU on FBG, %ΔFBG and serum INS in alloxan-induced diabetic rats

Effects of repeated single daily dosing of alloxan-induced diabetic rats on the 1st and 15th day FBG and serum INS concentrations with 50-200 mg/kg of *HU* are shown in Table 2. Treatment with alloxan resulted in sustained significant ($p<0.0001$) reductions in the circulating INS with concomitant significant ($p<0.0001$) rise in the FBG levels in Groups II rats when compared to untreated normal (Group I) rats (Table 2). Treatment with 50-200 mg/kg of *HU* for 14 days, however, did not significantly ($p>0.05$) alter the serum INS levels despite significant ($p<0.05$, $p<0.001$ and $p<0.0001$) dose-related reductions in the FBG levels induced by *HU* treatment relative to the values obtained for untreated alloxan-induced diabetic (Group II) rats (Table 2).

Effect of 50-200 mg/kg of HU on liver glycogen and glucose-6-phosphatase levels in alloxan-induced diabetic rats

Alloxan treatment was observed to have caused significant ($p<0.0001$) reductions in the hepatic glycogen while causing significant elevation in glucose-6-phosphatase activity when compared to untreated normal (Group I) rats (Table 3). With repeated oral treatment with *HU*, there were significant ($p<0.05$, $p<0.001$ and $p<0.0001$) dose-related increases in the hepatic glycogen and concomitant reduction in glucose-6-phosphatase activity with the highest levels recorded in rats treated with 200 mg/kg/day of *HU* and with the values for both relatively comparable to that of glibenclamide (Table 3).

Table 1. Effect of 14 days of oral treatment with 50-200 mg/kg *HU* on average body weights and percentage weight changes (%ΔWt) in alloxan-induced diabetic rats

Group	Day 1 wt. (g)	Day 15 wt. (g)	%ΔWt
I	135.20 ± 1.60	147.20 ± 2.27	8.91 ± 1.57
II	138.20 ± 1.70	112.00 ± 4.41	-19.06 ± 2.28^{f}
III	137.50 ± 2.49	157.80 ± 2.69	14.86 ± 1.72^{c}
IV	137.70 ± 2.06	144.80 ± 2.66	5.38 ± 0.59^{a}
V	137.30 ± 1.80	144.70 ± 2.22	5.36 ± 1.16^{a}
VI	136.80±2.61	141.00 ± 3.22	3.02 ± 0.61^{a}

^{f} $p<0.0001$ Vs Group I, ^{a} $p<0.05$ vs Group II, ^{c} $p<0.0001$ vs Group II values. Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU*, Group V: Diabetic+100 mg/kg *HU*, Group VI: Diabetic+200 mg/kg *HU*

Table 2. Effect of 14 days of oral treatment with 50-200 mg/kg of *HU* on the 1st and 15th day FBG, %FBG changes (%ΔFBG) and serum insulin in alloxan-induced diabetic rats

Group	Day 1 FBG (g)	Day 15 FBG (g)	%ΔFBG	serum insulin (ng/dl)
I	56.33±1.26	57.17±2.69	1.36 ± 3.60	0.96 ± 0.04
II	255.20±1.89^{c+}	302.50±5.17^{c}	18.52 ± 1.30^{c}	0.35 ± 0.03^{f}
III	255.50±1.34^{c+}	149.20±4.	-41.64± 1.49^{f}	0.42 ± 0.04^{f}
IV	255.20±2.18^{c+}	207.00± 3.33^{e}	-18.89± 0.79^{e}	0.42 ± 0.02^{f}
V	257.30 ± 2.49^{c+}	191.70± 1.59^{e}	-25.03± 0.60^{e}	0.38 ± 0.03^{f}
VI	256.20 ± 3.21^{c+}	159.00± 7.24^{f}	-38.05± 2.06^{f}	0.34 ± 0.03^{f}

$^{c+}$ $p<0.0001$ Vs Group I, ^{f} $p<0.0001$ Vs Group I, ^{e} $p<0.001$ Vs Group II, and ^{f} $p<0.0001$Vs Group II. Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU*, Group V: Diabetic+100 mg/kg *HU*, Group VI: Diabetic+200 mg/kg *HU*

Table 3. Effect of 50-200 mg/kg of *HU* on liver glycogen and glucose-6-phosphatase levels in alloxan-induced diabetic rats

Group	liver glycogen (mg/g)	glucose-6-Phosphatase (U/mg of protein)
I	5.78 ± 0.14	3.03 ± 0.25
II	3.34 ± 0.18^{c}	4.53 ± 0.08^{c+}
III	6.73 ± 0.17^{c}	2.48 ± 0.06^{e}
IV	4.07 ± 0.15^{a}	2.89 ± 0.08^{d}
V	4.80 ± 0.22^{b}	2.26 ± 0.08^{e}
VI	7.15 ± 0.19^{c}	2.04 ± 0.10^{f}

$^{c+}$ and $^{c-}$ represent significant increases and decreases at $p<0.0001$ Vs Group I. ^{d}$p<0.05$, ^{e} $p<0.001$, and ^{f} $p<0.0001$ vs Group II. Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* ,Group VI: Diabetic+200 mg/kg *HU*

Table 4. Effect of 50-200 mg/kg of *HU* treatment on serum TP, ALB, TG and TC in alloxan-induced diabetic rats

Group	TP (mg/dl)	ALB(mg/dl)	TG (mg/dl)	TC (mg/dl)
I	6.08 ± 0.15	3.70± 1.45	141.00±7.17	116.50±3.92
II	2.38±0.10^{c-}	1.43±0.07^{c-}	272.30±2.70^{c+}	253.00±3.76^{c-}
III	5.03±1.80^{c}	3.00±0.14^{c}	223.50±5.07^{e}	208.70±3.82^{e}
IV	3.08±0.09^{a}	1.82±0.06^{a}	233.20±7.10^{d}	221.70±6.33^{d}
V	3.86±0.12^{b}	2.60±0.10^{b}	206.30±3.07^{e}	186.70±4.18^{e}
VI	5.00±0.09^{c}	3.10±0.06^{c}	174.50± 4.19^{f}	156.50± 2.84^{f}

$^{c-}$ and $^{c+}$ represent significant decrease and increase at $p<0.0001$, respectively, relative to untreated normal (Group I) rats. ^{a,} ^{b} and ^{c} represent significant increases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, while ^{d,} ^{e} and ^{f} represent significant decrease at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, relative to untreated diabetic (Group II) values Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* , Group VI: Diabetic+200 mg/kg *HU*

Table 5. Effect of 50-200 mg/kg of *HU* treatment on the serum levels of AST, ALT, ALP and LDH in alloxan-induced diabetic rats

Group	AST(U/mg protein)	ALT(U/mg protein)	ALP(U/mg protein)	LDH(U/mg protein)
I	37.00 ± 1.75	58.50 ± 1.23	37.67 ± 4.35	288.30 ± 3.18
II	156.70 ± 4.65^{c+}	142.00 ± 5.87^{c+}	149.70 ± 5.57^{c+}	560.20± 7.44^{c}
III	67.00 ± 7.53^{e}	53.00 ± 5.02^{e}	57.00 ± 5.15^{e}	296.60 ± 6.83^{f}
IV	90.83 ± 2.59^{e}	57.33 ± 2.46^{e}	79.17 ± 2.71^{d}	351.50 ± 2.95^{d}
V	83.17 ± 2.75^{e}	53.33 ± 2.97^{e}	66.50 ± 4.40^{e}	336.90±10.30^{e}
VI	55.14 ± 2.66^{f}	33.50 ± 1.93^{f}	41.40 ± 2.79^{f}	255.80 ± 7.60^{f}

$^{c+}$ represents a significant increase at $p<0.0001$ relative to untreated normal (Group I) rats while ^{d,} ^{e} and ^{f} represent significant decreases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, relative to untreated diabetic (Group II) values Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* ,Group VI: Diabetic+200 mg/kg *HU*

Table 6. Effect of 50-200 mg/kg of *HU* treatment on hepatic tissue SOD, CAT, GSH and MDA in alloxan-induced diabetic rats

Group	SOD(U/mg protein)	CAT(U/mg protein)	GSH(U/mg protein)	MDA(nM/mg protein)
I	14.57 ± 1.10	7.68 ± 0.34	8.83 ± 0.47	0.67 ± 0.05
II	04.55 ± 0.36^{c}	03.63 ± 0.41^{c-}	01.55 ± 0.24^{c-}	02.13 ± 0.13^{c+}
III	24.70 ± 1.02^{c}	09.02 ± 1.17^{c}	12.73 ± 0.63^{c}	0.61 ± 0.10^{e}
IV	07.12 ± 0.38^{a}	05.30 ± 0.18^{a}	02.45 ± 0.15	02.10 ± 0.23
V	13.53 ± 0.89^{b}	07.48 ± 0.39^{b}	07.73 ± 0.61^{a}	00.59 ± 0.04^{e}
VI	20.50 ± 0.78^{c}	08.73 ± 0.27^{c}	11.50 ± 0.31^{b}	0.41 ± 0.04^{f}

$^{c-}$ and $^{c+}$ represent significant decrease and increase at $p<0.0001$, respectively, relative to untreated normal (Group I) rats. ^{a,} ^{b} and ^{c} represent significant increases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, while ^{e} and ^{f} represent significant decrease at $p<0.001$ and $p<0.0001$, respectively, relative to untreated diabetic (Group II) values Group I: normal, Group II: Diabetic untreated, Group III: Diabetic+glibenclamide, Group IV: Diabetic+50 mg/kg *HU* , Group V: Diabetic+100 mg/kg *HU* ,Group VI: Diabetic+200 mg/kg *HU.*

Effect of HU treatment on serum proteins (TP and ALB) and lipids (TG and TC) in alloxan-induced diabetic rats

Alloxan treatment resulted in significant ($p<0.0001$) reductions in the serum TP and ALB levels and concomitant significant ($p<0.0001$) elevations in the serum TG and TC when compared to the untreated normal (Group I) rats (Table 4). However, oral treatments with 50-200 mg/kg of *HU* significantly ($p<0.05$, $p<0.001$, $p<0.0001$) reversed the effect of alloxan in the treated rats in a dose-related fashion with the most significant effect recorded for the group treated with the highest dose of *HU* (Table 4).

Effect of HU treatment on serum AST, ALT, ALP and LDH in alloxan-induced diabetic rats

Table 5 represents the effect of 50-200 mg/kg of *HU* on the serum levels of AST, ALT and ALP in alloxan-induced diabetic rats following oral treatment with the extract for 14 days. Alloxan treatment resulted in significant ($p<0.0001$) elevations in the serum AST, ALT and ALP levels and these were significantly ($p<0.05$, $p<0.001$ and $p<0.0001$) reversed by repeated oral treatment with 50-200 mg/kg of *HU* in a dose-related fashion (Table 5).

Effect of HU treatment on liver SOD, CAT, GSH and MDA levels in alloxan-induced diabetic rats

Treatment of rats with 150 mg/kg of alloxan given intraperitoneally resulted in significant ($p<0.0001$) reductions in the hepatic SOD, CAT activities and GHS levels while at the same time significantly ($p<0.0001$) enhancing the activity of MDA relative to untreated normal (Group I) control (Table 6). These effects were significantly ($p<0.05$, $p<0.001$ and $p<0.0001$) reversed by oral treatment with 50-200

mg/kg *HU* dose dependently with the most significant improvement recorded for the group treated with the highest dose (200 mg/kg) of *HU* (Table 6).

DISCUSSION

Recent and accumulating reports from both preclinical and clinical studies showed that oxidative stress plays a central and important role in the onset and course of DM as well as in the development of its associated vascular and neurological complications (Hunt *et al*., 1988; Niedowicz and Daleke, 2005), with diversion of glycolytic intermediates into pathological pathways (Turk, 2010). Also, in DM, there is increased oxygen free radicals generation and disproportionately increased decline in antioxidant defense mechanism resulting in glucose oxidation, non-enzymatic glycation of proteins and subsequent oxidative degradation of cellular organelles and enzymes, increased lipid peroxidation, and rapid decline in pancreatic β-cells population (Domínguez *et al*., 1998; Ceriollo, 2006) with consequent progression and development of secondary diabetic complications such as retinopathy, nephropathy, neuropathy, and accelerated coronary artery disease (McGrowder, 2013).

In this study, type 1 DM was induced through the intraperitoneal injection of cold alloxan monohydrate in normal saline into Wistar rats. Alloxan is known to cause diabetes and oxidative/nitrosative stress after its intracellular accumulation in the pancreatic β-cells via the GLUT2 glucose transporters through ROS mechanism which results in pancreatic β-cell destruction (Lenzen, 2008a). In addition, pancreatic β-cells are known to be highly sensitive and susceptible to oxidative stress as the intrinsic antioxidative defense mechanisms of pancreatic β-cells are weak and are easily overwhelmed by redox imbalance from reactive oxygen and nitrogen species with attendant deleterious consequences such as lipid peroxidation, protein oxidation, DNA damage (Lenzen, 2008b). Interference of reactive species [such as superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), toxic hydroxyl radicals ($^{\cdot}OH$), singlet oxygen, nitric oxide, and peroxynitrite] with signal transduction pathways contributes significantly to β-cell dysfunction and death (Lenzen, 2008b; Djordjevic *et al.*, 2004). In *in vivo* experimental models, tissue oxidative stress markers such as SOD, CAT and GSH are useful and reliable markers of antioxidant status while MDA is a sensitive and reliable marker for lipid peroxidation (Kumar *et al*., 2010; Feillet-Coudray *et al.*, 1999). Similarly, the integrity of hepatocytes is reliably assessed by levels of the serum liver enzyme markers such as AST, ALT and ALP (Moss and Henderson, 1999) and often these marker enzymes are elevated in DM (Rao *et al.*, 1989). In the current study, oxidative stress were reliably induced with alloxan in the treated rats as indicated by marked reductions in the hepatic tissue SOD, CAT, GSH and marked elevation in hepatic tissue MDA levels and concomitant elevations in the serum AST, ALT and ALP levels which are in complete agreement with results of other studies (Golberg *et al*., 1977; Stanely and Menon, 2001; Ezekwesili, 2012; Rajaram, 2013). Reversal in the hepatic tissue levels of these oxidative markers and hepatic enzyme markers following repeated oral treatment with 50-200 mg/kg/day of *HU* strongly indicate the effective therapeutic role of *HU* in attenuating oxidative stress associated with type 1 DM which was probably mediated via free radical scavenging activities and improving glutathione status in the liver tissues. Consequently, this result is in consonance with that of an earlier study which reported the *in vitro* anti-oxidant activity of *HU* (Adeneye *et al.*, 2011). Similarly, significant reductions in the serum liver enzyme markers, total cholesterol, triglyceride, and concomitant significant elevations in the serum total protein and albumin levels are suggestive of the hepatoprotective potential of *HU* against oxidative stress induced by alloxan since these biochemical parameters were normalized by *HU* treatments.

Alloxan, as a thiol reagent, selectively inhibits glucose-induced insulin secretion through its ability to inhibit β-cell glucose sensor, glucokinase, an essential rate-limiting step glucose metabolic enzyme (Lenzen, 2008b). The alloxan treated animals exhibited a decrease in hepatic glycogen content which may be due to enhancements in the glucose-6-phosphatase activity and deactivation/inhibition of glucokinase activity (Shirwaikar *et al*., 2004). Glucose-6-phosphatase is known to catalyze the final step of glucose production by liver and kidney and its activity is often elevated in DM (Liu *et al.*, 1994; Clore *et al.*, 2000). Similarly, in type 1 DM, LDH activity is often significantly enhanced, resulting in lactic acidosis as a metabolic complication of type 1 DM (Zappacosta *et al.*, 1995; Raju *et al.*, 2001). The fact that glucose-6-phosphatase activity was significantly enhanced in the rats treated with alloxan in this study shows that our result is in accord with report of Liu *et al*. (1994) and Clore *et al.* (2000). Thus, the profound control of hyperglycemia coupled with increased hepatic glycogen content, decreased glucose-6-phosphatase and reduced lactate dehydrogenase activities in the *HU*-treated rats as recorded in this study suggest that the glycemic control achieved by *HU* was probably mediated via decreased release of glucose from hepatic tissue glycogen and increased hepatic glycogen deposition/storage due to decreased glucose-6-phosphatase activity through inhibition of glycolysis. Also, literature has it that an increase in hepatic

glycogen is often mediated through the activation of glycogen synthase for which the substrate glucose-6-phosphate could have been readily provided by increased hexokinase activity (Shirwaikar *et al.*, 2004; Bouche *et al.*, 2004; Lawrence and Roach, 1997). Again, significant reduction in glucose-6-phosphatase activity following treatment with *HU* coupled with insignificant alterations in the serum insulin levels lends support to the earlier report that *HU* achieves glycemic control via increased peripheral glucose utilization (Adeneye and Adeyemi, 2009b). Thus, the current study has further provided an insight into the enhanced peripheral glucose utilization mechanism of *HU* in alloxan-induced diabetic rats.

Another significant finding of this study is the improvement in the average body weight and percentage weight changes following repeated oral treatment with 50-200 mg/kg/day of *HU*. Results of the current study showed that untreated alloxan-induced diabetic rats manifested significant weight loss, hypercholesterolemia and hypertriglyceridemia which are in concordance with earlier studies that reported alloxan to cause significant weight loss with metabolic derangements such as hyperglycemia, dyslipidemia, hyperketonemia, lactic acidosis, *etc.* (Abdulrahman *et al.*, 2013). The fact that these metabolic alterations were restored to near normal levels following *HU* treatment also suggest that these metabolic re-arrangements by *HU* treatment could probably have accounted for the improvement in the body weight changes seen in the *HU*-treated rats.

Overall, results of this study show a positive correlation between chronic hyperglycemia and oxidative stress in alloxan-induced diabetic rats and both the hyperglycemia and oxidative stress were profoundly ameliorated with *HU* treatment via enhanced hepatic glycogen deposition mediated via decreased hepatic glucose-6-phosphatase activity and improvement in antioxidant/free radicals scavenging activities, respectively. Thus, this study provides further insight into the antidiabetic and antioxidant mechanisms of *HU* in experimental type 1 DM.

REFERENCES

Abdulrahman, M.M., El-Hefnawy, M.H., Aly, R.H., *et al*. (2013). Metabolic effects of honey in type 1 diabetes mellitus: a randomized crossover pilot study. *Journal of Medicinal Food* 16(1): 66-72.

Adeneye, A.A. and Adeyemi, O.O. (2009a). Hypoglycemic effects of the aqueous seed extract of *Hunteria umbellata* in normal and glucose- and nicotine-inducedhyperglycemic rats. *International Journal of Applied Research and Natural Products* 2(1): 9-18.

Adeneye, A.A. and Adeyemi, O.O. (2009b). Further evaluation of the antihyperglycemic effect of *Hunteria umbellata* (K. Schum.) Hallier f. seed extract in experimental diabetes. *Journal of Ethnopharmacology* 126(2): 238-243.

Adeneye, A.A., Adeyemi, O.O., Agbaje, E.O. (2010). Anti-obesity and antihyperlipidemic effect of *Hunteria umbellata* seed extract in experimental hyperlipidemia. *Journal of Ethnopharmacology* 130(2): 307-314.

Adeneye, A.A., Crooks, P.A., Fadhel-Albayati, Z., *et al*. (2013). Antihyperglycemic profile of erinidine isolated from *Hunteria umbellata* seed. *African Journal of Traditional, Complementary and Alternative Medicine* 10(2): 189-202.

Adeneye, A.A., Crooks, P.A., Miller, A-F., *et al*. (2012). Isolation and structure elucidation of a new indole alkaloid, erinidine, from *Hunteria umbellata* seeds. *Pharmacologia* 3(7): 204-214.

Adeneye, A.A., Sofidiya, M.O., Adenekan, O.S. (2011). Anti-inflammatory and antioxidant activities of *Hunteria umbellata* seed fractions. *Pharmacologia* 2(6): 165-171.

Baginsky, E.S., Foa, P.P., Zak, B. (1992). In: Bergmeyer, H.U. and Gawehn, K. (eds). *Methods of enzymatic analysis*, 2nd edition. Academic Press, New York, pp. 876-880.

Bouche, C., Serdy, S., Kahn, C.R., *et al*. (2004). The cellular fate of glucose and its relevance in type 2 diabetes. *Endocrine Reviews* 25: 807-830.

Carroll, N.V., Longley, R.W., Roe, J.H. (1956). The determination of glycogen in liver and muscle by use of anthron reagent. *Journal of Biological Chemistry* 220: 583-593.

Ceriollo, A. (2006). Oxidative stress and diabetes-associated complications. *Endocrine Practice* 12(Suppl1): 60-62.

Chattopadhyay, R.R., Sakar, S.K., Ganguly, S., *et al*. (1992). Effect of leaves of *Vinca rosea* Linn. on glucose utilization and glucose deposition by isolated rat hemi-diaphragm. *Indian Journal of Physiology and Pharmacology* 36: 137-138.

Clore, J.N., Stillman, J., Sugerman, H. (2000). Glucose-6-phosphatase flux *in vitro* is increased in type 2 diabetes. *Diabetes* 49(6): 967-974.

Djordjevic, A., Spasic, S., Jovanovic-Galovic, A., *et al.* (2004). Oxidative stress in diabetic pregnancy: SOD, CAT and GSH-Px activity and lipid peroxidation products. *Journal of Maternal, Fetal and Neonatal Medicine* 16: 367-372.

Domínguez, C., Ruiz, E., Gussinye, M., *et al*. (1998). Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. *Diabetes Care* 21(10): 1736-1742.

Doumas, B.T., Watson, W.A., Biggs, H.G. (1971). Albumin standard and the measurement of serum

albumin and bromocresol green. *Clinica Chimica Acta* 31: 131-137.

Ezekwesili, C.N., Ogbunugafor, H.A., Ezekwesili-Ofili, J.O. (2012). Antidiabetic activity of aqueous extract of *Vitex dodiana* leaves and *Cinchona calisaya* bark in alloxan-induced diabetic rats. *International Journal of Tropical Disease and Health* 2(4): 290-300.

Falodun, A., Nworgu, Z.A.M., Ikponmwonsa, M.O. (2006). Phytochemical components of *Hunteria umbellata* (K. Schum.) and its effect on isolated non-pregnant rat uterus in oestrus. *Pakistani Journal of Pharmaceutical Sciences* 19(3): 256-258.

Feillet-Coudray, C., Rock, E., Coudray, C. (1999). Lipid peroxidation and antioxidant status in experimental diabetes. *Clinica Chimica Acta* 284: 31-43.

Frier, B.M. and Fisher, M. (2010). Diabetes mellitus. In: Colledge, N.R., Walker, B.R., Ralston, S.H. (eds.). *Davidson's Principles and Practice of Medicine*, 21st edition. Churchill Livingstone Elsevier, Edinburg, pp. 795-834.

Goldberg, D.M., Martin, J.V., Knight, A.H. (1977). Elevation of serum alkaline phosphate activity and related enzymes in diabetes mellitus, *Clinical Biochemistry* 10(1): 8-11.

Gupta, M.P., Solis, N.G., Avella, M.E., *et al*. (1984). Hypoglycemic activity of *Neurolaena lobata*. *Journal of Ethnopharmacology* 10: 323-327.

Herbert, V., Lau, K.S., Gottlieb, C.W., *et al.* (1965). Coated charcoal immunoassay of insulin. *Journal of Clinical Endocrinology and Metabolism* 25: 375-384.

Hunt, J., Dean, R.T., Wolff, S.P. (1988). Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes and ageing. *Biochemical Journal* 256: 205-212.

Igbe, I., Omogbai, E.K.I., Ozolua, R.I. (2009). Hypoglycemic activity of aqueous seed extract of *Hunteria umbellata* in normal and streptozotocin-induced diabetic rats. *Pharmaceutical Biology* 47(10): 1011-1016.

Iwalewa, E.O., Adewale, I.O., Taiwo, B.J., *et al*. (2008). Effects of *Harungana madagascariensis* stem bark extract on the antioxidant markers in alloxan-induced diabetic and carrageenan-induced inflammatory disorders in rats. *Journal of Complementary and Integrative Medicine* 5(1): 1-18.

Kakkar, P., Dos, B., Viswanathan, P.N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics* 21: 130-132.

Kaneto, H., Katakami, N., Kawamori, D., *et al.* (2007). Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxidant and Redox Signaling* 9: 355-366.

Kaur, G., Jabbar, Z., Anthar, M., *et al.* (2006). *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. *Food and Chemical Toxicology* 44: 984-993.

Kingsley, S.R. and Frankel, S.J. (1939). The determination of serum total protein, albumin and globulin by the biuret reaction. *Journal of Biological Chemistry* 128: 131-137

Kumar, R.P., Sujatha, D., Saleem, T.S.M., *et al*. (2010). Potential antidiabetic and antioxidant activities of *Morus indica* and *Asystasia gangetica* in alloxan-induced diabetes mellitus. *Journal of Experimental Pharmacology* 2: 29-36.

Lawrence, J.C. and Roach, P.J. (1997). New insights into the role and mechanism of glycogen synthase activation by insulin. *Diabetes* 46: 541-547.

Lenzen, S. (2008a). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 51(2): 216-226.

Lenzen, S. (2008b). Oxidative stress: vulnerable beta-cell. *Biochemical Society Transactions* 36(Pt 3): 343-347.

Liu, Z., Barrett, E.J., Dalkin, A.C., *et al*. (1994). Effect of acute diabetes on rat hepatic glucose-6-phosphatase activity and its messenger RNA level. *Biochemical and Biophysical Research Communications* 205(1): 680-686.

Maiese, K., Chong, Z.Z., Shang, Y.C. (2007). Mechanistic insights into diabetes mellitus and oxidative stress. *Current Medicinal Chemistry* 16(16): 1729-1738.

McGrowder, D.A., Anderson-Jackson, L., Crawford, T.V. (2013). Biochemical evaluation of oxidative stress in type 1 diabetes. In: Escher, A.P. and Li, A. (eds). *Diabetes type 1*. InTech Publishing Co., Crotia, pp. 223-248. DOI: 10.5772/52836.

Moss, D.W. and Henderson, A.R. (1999). Clinical enzymology. In: Burtis, C.A. and Ashwood, E.R. (eds). *Tietz's Textbook of Clinical Chemistry*, 3rd edition. WB Saunders, Philadelphia, pp. 617-721.

Niedowicz, D. and Daleke, D. (2005). The role of oxidative stress in diabetic complications. *Cell Biochemistry and Biophysics* 43: 289-330.

Petersen, K.F., Befroy, D., Dufour, S., *et al.* (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300(5622): 1140-1142.

Rajaram, K. (2013). Antioxidant and antidiabetic activity of *Tectona grandis* Linn. in alloxan
induced albino rats. *Asian Journal of Pharmaceutical and Clinical Research* 6(Suppl 3): 174-177.

Rahul, S., Sanjay, K., Abhay, K.S. (2006). Antidiabetic potential of *Butea monosperma* in rats. *Fitoterapia* 77: 86-90.

Raju, J., Gupta, D., Rao, A.R., *et al*. (2001). *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. *Molecular and Cellular Biochemistry* 224(1-2): 45-51.

Rao, G.M., Morghom, L.O., Kabur, M.N., *et al*. (1989). Serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in diabetes mellitus. *Indian Journal of Medical Sciences* 43(5): 118-121.

Robertson, R.P. (2004). Chronic oxidative stress as central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *Journal of Biological Chemistry* 279(41): 42351-42354.

Robertson, R.P., Harmon, J., Tran, P.O., *et al.* (2003). Glucose toxicity in β-cells: Type 2 diabetes, good radicals gone bad, and glutathione connection. *Diabetes* 52(3), 581-587.

Shirwaikar, A., Rajendran, K., Kumar, C.D., *et al*. (2004). Antidiabetic activities of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. *Journal of Ethnopharmacology* 91: 171-175.

Sinha, A.K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry* 47: 389-394.

Stanely, M.P.P. and Menon, V.P. (2001). Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytotherapy Research* 15: 213-218.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. (2002). Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 25(Suppl.l): S5-S20.

Trinder, P. (1969). Determination of blood glucose using 4-aminophenzone as oxygen acceptor. *Journal of Clinical Pathology* 22: 246-248.

Turk Z. (2010). Glycotoxines, carbonyl stress and relevance to diabetes and its complications.
Physiological Research 59: 147-156.

United States National Institutes for Health. (1985). Publication no. 85-23.

Venogopal, P.M., Prince, P.S.M., Pari, L. (1998). Hypoglycemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology* 61: 1-7.

Wild, S., Roglic, G., Green, A., *et al.* (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27: 1047-1053.

Wroblewski, F. and LaDue, J.S. (1955). Lactic dehydrogenase activity in blood. *Proceedings of Society for Experimental Biology and Medicine* 90: 210-213.

Zappacosta, B., De Sole, P., Rossi, C., *et al*. (1995). Lactate dehydrogenase activity of platelets in diabetes mellitus. *European Journal of Clinical Chemistry and Clinical Biochemistry* 33(8): 487-489.

Niger. J. Physiol. Sci. 29(June 2014) 007 –010
www.njps.com.ng

Niger. J. Physiol. Sci.

Review

Vascular Effects of Histamine

*** Ebeigbe Anthony B. and Talabi Olufunke O.**

Department of Physiology, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.

Summary: Four subtypes of receptors (H_1, H_2, H_3 and H_4) mediate the actions of histamine. In the vascular wall, the effects of histamine are mediated via H_1 and H_2 receptors and the actions are modulated by H_3 receptor subtype located on presynaptic neurones. Alterations in vascular responses to histamine are associated with experimental as well as a human form of hypertension, suggesting a role for histanine in cardiovascular regulation.

Keywords*:* Histamine, Vascular smooth muscle, Endothelium

**Address for correspondence:* ebeigbe@fulbrightmail.org, Tel:+234 805 850 3192, Fax: +1 315 702 1892

Manuscript Accepted: April, 2014

INTRODUCTION

Histamine or β-aminoethylimidazole is a chemical mediator that was first detected as uterine stimulant in extracts of ergot. It was also observed to stimulate a host of smooth muscles and to possess vasodepressor action (Dale and Laidlaw (1910). Histamine which is a diamine derivative of histidine is produced by the action of the enzyme histidine decarboxylase (Smuda and Bryce, 2011).

Endogenous histamine is a classical inflammatory and immunological mediator mainly produced by mast cells and basophils and plays a role in allergic response, regulation of gastric-acid secretion, neurotransmittion in the central nervous system and cardiovascular function. Four subclasses of receptors (H_1, H_2, H_3 and H_4) mediate the actions of histamine (Black et al, 1972; Arrang et al, 1983; Leurs et al, 1995; Rangachari, 1998). In general, activation of H_1 receptors (Ash and Schild, 1966) results in vasoconstriction while H_2-receptor activation (Black et al, 1972) mediates vasodilation. H_3 receptors (Arrang et al,1983) are described as modulators of histamine synthesis and release in the CNS, therefore, primarily function in modulation of neurotransmitter. H_4 receptors possess a limited expression; they are expressed in haemopoetic cells, involved in immunne response and are targets of particular interest in immunomodulatory therapies (Smuda and Bryce, 2011).

VASCULAR HISTAMINE

The vascular walls of various animal species have been reported to contain large amounts of histamine located in mast cell and non-mast cell stores (El Ackad and Brody, 1975). Also, coronary arteries of some patients with coronary artery disease have been reported to be hyperresponsive to histamine and to contain significantly higher concentrations of histamine (Kalsner and Richards, 1984). Mast cells present in post capillary venules also secrete histamine which induce protein leakage and edema formation

ACTIVATION OF HISTAMINE RECEPTORS

The actions of histamine are mediated via specific receptors on the cell embrane. The four subtypes of histamine receptors are typical G protein-coupled receptors (Rangachari, 1998). In general, activation of vascular H_1 and H_2 receptors elicits (respectively) vasoconstriction and vasodilatation (Black et al, 1972; Ebeigbe et al, 1989); vasodilation is, by far, the more predominant effect of histamine in humans. The H_1 and H_2 receptors mediating vasodilation are distributed throughout the resistance vessels in most vascular beds. Activation of either H_1 or H_2 subtype of histamine receptor can elicit maximal vasodilation, but the responses differ in their sensitivity to histamine, in duration of the effect, and in the mechanism of their production (Hudgins and Weiss, 1968; Ebeigbe et al, 1989; Leurs et al, 1995).

H_2 receptors are located mainly on vasuclar smooth muscle cells and the vasodilator effects produced by their stimulation are mediated by cyclic Adenosine monophosphate (cAMP); H_1 receptors reside mainly on endothelial cells, and their stimulation lead to the formation of local vasodilator substance called

Endothelium derived relaxing factor (EDRF) which has been identified as Nitric Oxide (NO). The EDRF diffuses from endothelial cells to vascular smooth muscle cells and therein activates the enzyme guanylate cyelase, which increases the level of cyclic guanosine monophosphate (cGMP) that leads subsequently, to the relaxation of the vascular smooth muscle (Furchgott and Zawadzki, 1980; Moncada et al, 1989).

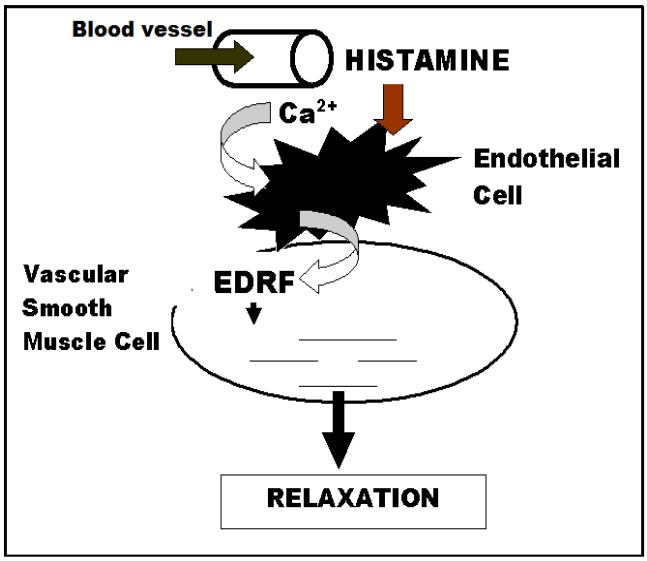

Fig. 1. Schematic representation of endothelium-derived relaxant factor (EDRF)-induced vascular smooth muscle relaxation in response to histamine (released from vascular wall) action on the endothelial cell.

The basal formation of nitric oxide maintains a moderate but significant vasodilation in the systemic resistance vessels. When blood flow in the conduit arteries is increased, there is an augemented endothelial formation of nitric oxide, eliciting flow-dependent vasodilation. Histamine has been widely reported to stimulate endothelial nitric oxide formation in a number of vascular beds (Van De Voorde and Leusen, 1983; Schoeffter and Godfraind, 1988).

Presynaptic H_3 receptors play a role in pathophysiology of cardiac ischemia. H_3 receptors in the heart become activated in the early phase of myocardial ischemia characterized by an increased histamine spillover (Gothert et al 1995). H_3 receptor in the central nervous system appear to be of importance in the control of vascular functions (Schlicker et al 1994). It is found either on histaminergic neurons of the CNS (Autoreceptors) or on the non-histaminergic neurons of the CNS (heteroreceptors). The vascular H_3 receptors appear to play some yet unidentified role in hypertension (Schlicker et al 1994).

H_4 receptor which has recently been characterised as the immune system histamine receptor (Zampeli and Tiligada, 2009) has a regulatory role in the immune system, is involved in dendritic cell activation and T cell differentiation.

VASOACTIVE EFFECTS OF HISTAMINE

The vascular effects of histamine are routinely studied *in vitro*, on ring preparations of isolated blood vessels (Ebeigbe et al, 1983; Schoeffter and Godfraind, 1988; Obiefuna et al, 1991). In the absence of active tone, histamine elicits contraction of vascular smooth muscles (Van de Voorde and Leusen, 1983; Ebeigbe and Cabanie, 1992). The relaxant effect of histamine, however, is usually observed in precontracted blood vessels and is more marked in the presence of an H_1-receptor antagonist (Van de Voorde and Leusen, 1983; Schoeffter and Godfraind, 1988; Ebeigbe and Cabanie, 1992).

At the capillary level, histamine distends the vessel wall to exert inflammatory reactions such as extravasation of blood content. In contrast, the muscular arteries, such as the coronary and mesenteric arteries are constricted by histamine. In addition to the vasomotor action, histamine has been shown to promote gene expression in smooth muscle cells. (Sasaguri and Tanimoto, 2004). Hudgins and Weiss (1968) demonstrated that the histamine-induced contraction of rabbit aorta is dependent to a large extent upon Ca^{2+} entry from the extracellular space.

TRANSMURAL NERVE STIMULATION

In many isolated blood vessels, transmural nerve stimulation elicits contractile responses due to release of noradrenaline from adrenergic nerve terminals (Vanhoutte et al, 1981). However when a vessel is precontracted using various agonists (e.g. noradrenaline, 5-HT, Angiotensin II), transmural nerve stimulation results in frequency-dependent relaxation responses. This Ca^{2+}-dependent relaxation response is presumed to be mediated, at least in part, by the release of histamine (Ebeigbe et al, 1983; Gantzos et al, 1983).

HISTAMINE AND VASCULAR DISEASE

Histamine has been reported to play some role in mediating some cardiovascular diseases: the coronary arteries of come cardiac patients are hyperreactive and contain large stores of histamine (Kalsner and Richards,1984). Contractile responses to histamine are enhanced in vessels from atherosclerotic humans (Ginsburg et al, 1981); reduced endothelium-dependent relaxation responses to histamine have been reported in various animal (Fig. 2) hypertensive models (Lockette et al, 1986; Obiefuna et al, 1991), as well as a human form of hypertension (Ebeigbe and Cabanie, 1991; 1992). Also, rings of isolated human epigastric arteries from pregnancy-induced hypertensive women display modest but significantly greater sensitivity to histamine (Fig. 3) and are more susceptible to H_1 receptor blockade (Ebeigbe and Cabanie, 1991; 1992).

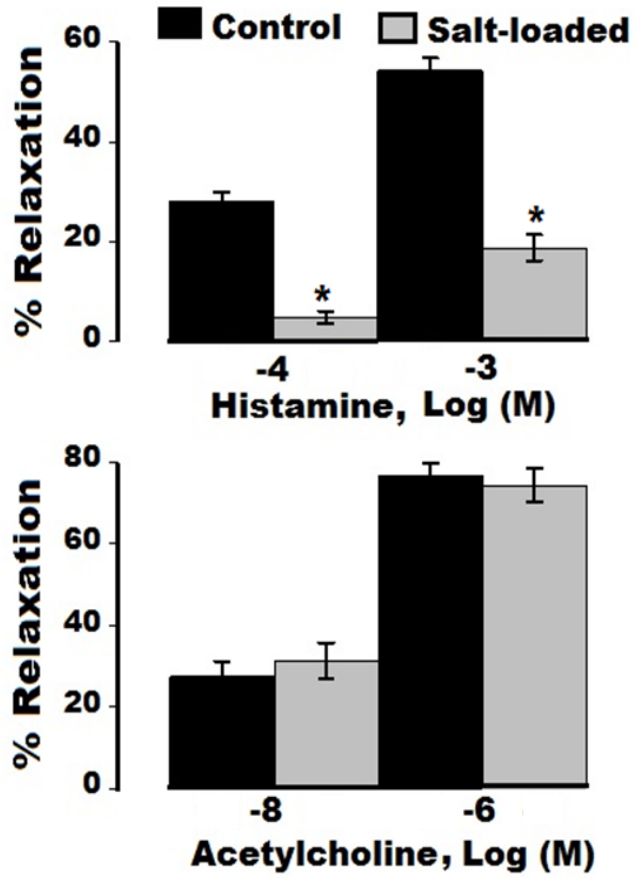

Fig. 2. Relaxation responses of isolated aortae from control and salt-loaded rats to histamine and acetylcholine. The relaxation responses to histamine, but not those to acetylcholine, were significantly (*p<0.05) diminished following salt-loading. (Adapted from Obiefuna, Sofola & Ebeigbe, 1991).

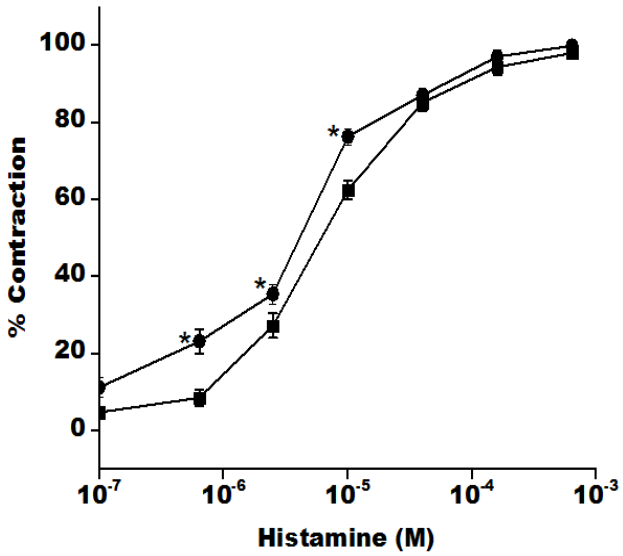

Fig. 3. Dose-response curves to histamine in epigastric arterial rings from control (n = 6, square) and pregnancy-induced hypertensive (n = 5, circle) patients. Asterisks denote significant difference from control values.

In conclusion, histamine, released from blood vessel wall or mast cells, influences vascular smooth muscle reactivity either directly or indirectly via stimulating endothelial cells. Alterations in histamine actions have implication for some cardiovascular disorders.

Acknowledgements
This work was supported, in part, by grants from Institute Henri Beaufour, Le Plessis Robinson, France and the University of Benin Research and Publications Committee.

REFERENCES

Arrang J.M., Garbarg M., Schwartz J.C. (1983): Autoinhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature (lond.)* **302:**832-837

Ash A.S and Schild H.O. (1966): Receptors mediating some actions of histamine. *Br. J. Pharmacol. Chemother*. **27(2):** 427-439

Black J.W., Duncan W.A.M., Durant C.J., Ganellin C.R. and Parsons M.E. (1972): Definition and antagonism of histamine H_1 receptors. *Nature* **236:** 385-390

Dale H.H. and Laidlaw P.P. (1910). The physiological action of beta-iminazolylethylamine. *J. Physiol.* **41:**318-344.

Ebeigbe A.B. and Cabanie M. (1991): In vitro vascular effects of cicletanine in pregnancy-induced hypertension. *Brit. J. Pharmacol.* **103:** 1992-1996

Ebeigbe A.B. and Cabanie M. (1992). Responses of isolated human epigastric arteries to histamine. J *Auton Nerv Syst*. **39**: 201-210.

Ebeigbe A.B., Cabanie M. and Godfraind T. (1989): Effects of Cicletanine on histamine-induced contractions of isolated rabbit mesenteric arteries. *Fundam. Clin. Pharmacol.* **3:** 223-235

Ebeigbe A.B., Gantzos R.D. and Webb R.C. (1983): Relaxation of rat tail artery to electrical stimulation. *Life Sci.* **33:** 303-309

El-Ackad T.M and Brody M.J. (1975): Evidence for non-mast cell histamine in the vascular wall. *Blood Vessels* **12:** 181-191

Furchgott R.F. and Zawadzki J.V. (1980). The Obligatory Role of Endothelial cell in the relaxation of Arterial Smooth Muscle by acetylcholine. *Nature*. **288**: 273-276

Gantzos R.D., Ebeigbe, A.B. and Webb R.C. (1983). Ca^{2+}, histamine antagonists and relaxation to electrical impulses in dog coronary artery. *Eur. J. Pharmacol*. 89: 287-291.

Ginsburg R., Bristow M.R., Kantrowitz N., Baim D.S. and Harrison D.C. (1981): Histamine provocation of clinical coronary artery spasm: implications concerning pathogenesis of variant angina pectoris. *Am. Heart. J.* **102:**819

Hudgins P.M. and Weiss G.B. (1968): Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J Pharmacol Exp Ther.* **159:** 91-97

Kalsner S. and Richards R. (1984): Coronary arteries of cardiac patients are hyperactive and contain

stores of amines: a mechanism for coronary spasm. *Science* **233:** 1435

Leurs R., Smit M.J., and Timmerman H. (1995): Molecular pharmacological aspects of histamine receptors. *Pharmacol. Ther.* **66:** 413-463

Lockette W., Otsuha Y. and Carretero O. (1986): The loss of endothelium-dependent relaxation in hypertension. *Hypertension* **8(2):** 61-66

Moncada S, Palmer R.M. and Higgs E.A. (1989). *Biochem. Pharmacol.* **38**:1709-15. Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication.

Obiefuna P.C.M., Sofola O.A. and Ebeigbe A.B. (1991): Dietary Salt-loading attenuates endothelium-dependent relaxation in responses to histamine but not to acetylcholine in rat aortic rings. *Exper. Physiol.* **76:** 135-138

Rangachari P.K. (1998). The fate of released histamine: reception, response and termination. *Yale Journal of Biol. Med.* **71**: 173-182.

Sasaguri Y. and Tanimoto A. (2004): Role of Macrophage-derived Histamine in Atherosclerosis-chronic participation in the inflammatory response. *Journal of atherosclerosis and thrombosis.* **11(3):** 122-130

Schlicker E., Malinowska B., Kathmann M. and Gothert M. (1994): Modulation of neurotransmitter release via histamine H_3 heteroreceptors. *Fundam. Clin. Pharmacol.* **8(2):** 128-137

Schoeffter P. and Godfraind T. (1988): Antihistaminic property of cicletanine in human isolated coronary arteries. *Drugs Exp. Clin. Res.* **14:** 159-165

Smuda C. And Bryce P.J. (2011): New Developments in the Use of Histamine and Histamine Receptors. *Curr. Allergy Asthma Rep*. **11(2):** 94–100.

Van de Voorde J. and Leusen I. (1983): Role of the endothelium in the vascular response of rat thoracic aorta to histamine. *Eur. J. Pharmacol.* **87:** 113-120

Vanhoutte P.M., Verbeuren T.J. and Webb R.C. (1981): Local modulation of the adrenergic neuroeffector interaction in the blood vessel wall. *Physiol. Rev.* **61:**151-247

Zampeli E. and Tiligada E. (2009): The role of histamine H_4 receptor in immune and inflammatory disorders. *Br. J. Pharmacol.* **157(1):** 24–33.