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

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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

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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

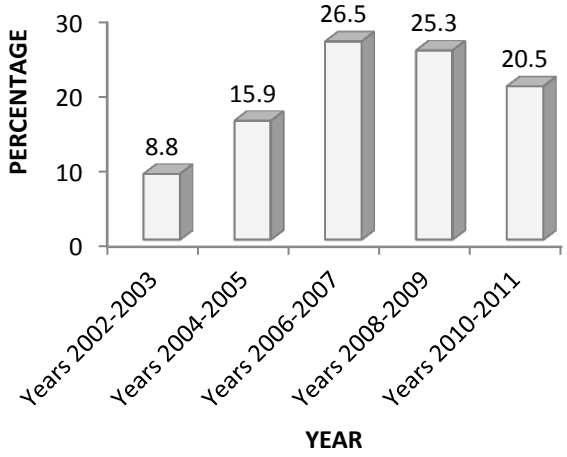

Figure 1: Two-yearly incidence of Newborn Brachial Plexus Palsy in the University College Hospital Ibadan over a ten year period

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.

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Role of Adrenergic Receptors in Glucose, Fructose and Galactose-Induced Increases in Intestinal Glucose Uptake in Dogs

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Summary: The study investigated the role of adrenergic receptors in glucose, fructose-, and galactose- induced increases in intestinal glucose uptake. Experiments were carried out on fasted male anaesthetized Nigerian local dogs divided into seven groups (with five dogs per group). Group I dogs were administered normal saline and served as control. Dogs in groups II, III and IV were intravenously infused with glucose (1.1 mg/kg/min), fructose (1.1 mg/kg/min) and galactose (1.1 mg/kg/min) respectively. Another three groups, V, VI and VII were pretreated with prazosin (0.2mg/kg), propranolol (0.5mg/kg) or a combination of prazosin (0.2mg/kg) and propranolol (0.5mg/kg) followed by glucose infusion, frutose infusion or galactose infusion respectively. Through a midline laparatomy, the upper jejunum was cannulated for blood flow measurement and blood samples were obtained for measurement of glucose content of the arterial blood and venous blood from the upper jejunal segment. Glucose uptake was calculated as the product of jejunal blood flow and the difference between arterial and venous glucose levels (A-V glucose). The results showed that pretreatment of the animal with prazosin had no effect on glucose and galactose induced increases in glucose uptake. However, pretreatment with propranolol completely abolished glucose, fructose and galactose-induced increases in intestinal glucose uptake. Prazosin also significantly reduced galactose-induced increase in intestinal glucose uptake. The results suggest that the increases in intestinal glucose uptake induced by glucose and fructose are mediated mostly by beta adrenergic receptors while that of galactose is mediated by both alpha and beta adrenergic receptors.

Keywords*:* Hexoses administration, Adrenergic receptors, Glucose uptake, Dog.

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INTRODUCTION

In previous studies, it was shown that the gastrointestinal tract (g.i.t) plays a role in glucose homeostasis. Thus, the g.i.t takes up large quantity of glucose from circulation following hyperglycemia induced by catecholamines (Grayson and Oyebola, 1983; Oyebola and Durosaiye, 1988; Alada and Oyebola, 1996; Oyebola et al, 2011); nicotine (Grayson and Oyebola, 1985); cow's urine concoction (Oyebola, 1982); glucagon (Alada and Oyebola, 1996); glucose (Alada and Oyebola, 1996) and diabetes mellitus (Alada et al; 2001). In addition, the g.i.t releases glucose into the blood stream in response to insulin induced hypoglycaemia. Recently, Salman et al; (2014) also showed that the canine intestine increased its glucose uptake significantly followings hyperglycaemia induced by glucose, fructose or galactose.

Although, Salman et al; (2014) did not report on the mechanisms of the increased intestinal glucose uptake in response to hyperglycaemia induced by glucose, fructose or galactose, Grayson and Oyebola (1983) and Oyebola et al; (2011) had reported the involvement of alpha and beta adrenergic receptors in catecholamine induced increases in intestinal glucose uptake in dogs and rabbits respectively. Also, Alada and Oyebola (1997) reported that the increase in intestinal glucose uptake caused by glucagon or glucose is mediated through beta adrenergic receptors. It is however not clear if adrenergic receptors are involved in the increases in intestinal glucose uptake induced by fructose or galactose induced hyperglycaemia. The present study was therefore designed to investigate the role of adrenergic receptors in intestinal glucose uptake increases induced by hexoses such as glucose, fructose and galactose in the dog.

MATERIALS AND METHODS

Male dogs weighing between 9-16 kg were used for the study. Each animal was fasted for 18-24 hours before the start of the experiment. Anaesthesia was

induced by an intravenous injection of 30mg/kg – body weight of sodium pentobarbitone. Light anaesthesia was maintained with supplementary doses of sodium pentobarbitone as necessary. The animal was laid supine and firmly secured on the dissecting table. The trachea was intubated using a Y-piece cannula and the animal was allowed to breathe room air (temp. 25C) spontaneously. A cannula was placed in the carotid artery to monitor arterial blood pressure (BP) using a pressure transducer connected to a channel recorder (Ugo Basil). Cannulae were also placed in the right femoral vein and right femoral artery. The latter was advanced to the level of the superior mesenteric artery.

Through a midline laparatomy, the jejunum was identified and a vein draining the proximal segment of the jejunum was cannulated using a 1.8 mm (i.d) polyethylene tubing (P.E). The jejunal vein cannula was moved into an extra-corporeal position and a non crushing clamp was applied to its free end. At the end of the surgical procedure, sodium heparin 300 i.u was administered intravenously to prevent blood clotting. The abdomen was then closed in two layers with interrupted sutures.

Following surgery, a period of 60 min was allowed for stabilization in all animals. Blood pressure and jejunal segment blood flow were continuously monitored throughout the duration of the experiment. Arterial and venous blood samples for glucose estimation were obtained from the femoral and jejunal venous cannulae respectively. Jejunal blood flow was determined by timed collection of the effluent from the jejunal venous cannula as previously described (Alada and Oyebola, 1996). Blood glucose was determined by the glucose oxidase method (Trinder, 1969). Arterio-venous glucose difference was calculated as the difference between arterial and venous blood glucose concentrations while intestinal glucose uptake (mg/min) was calculated as the product of the arterio-venous glucose difference and jejunal blood flow per minute.

Experimental procedures

Group I: Pre-treatment with alpha adrenergic receptor blocker.

Five dogs were first given prazosin before glucose, fructose or galactose infusion. Each dog was injected i.v with prazosin, 0.2 mg/kg. Forty minutes was allowed for the drug to take effect. Then, basal recording of blood pressure, blood flow and collection of arterial and venous blood samples for glucose estimation were made. After the basal recordings and blood sample collection, glucose (1.1 mg/kg/min) infusion was given for twenty minutes. The blood pressure, blood flow, arterial and venous blood glucose were similarly monitored at intervals for 90 min during infusion and post-infusion observation period. The experiment was repeated in another two subgroups (with five dogs per subgroup) using fructose infusion (1.1 mg/kg/min) or galactose (1.1 mg/kg/min) in place of glucose.

Group II: Pretreatment with beta adrenergic receptor blocker.

Five dogs were also first injected with propranolol before glucose, fructose or galactose infusion. Each animal was given i.v injection of propranolol, 0.5 mg/kg. After forty minutes and basal recording of blood pressure, jejunal blood flow and sample collection for arterial and venous blood glucose, glucose (1.1 mg/kg/) infusion was administered intravenously for twenty minutes. Similar measurements to those used in group I were made. Another two subgroups (of five dogs per subgroup) were studied with propranolol pre-treatment but using fructose (1.1 mg/kg/min) or galactose (1.1 mg/kg/min) infusion instead of glucose.

Group III: Pre-treatment with combined alpha and beta adrenergic receptor blockers.

Five dogs were first given a combination of prazosin and propranolol before i.v infusion of glucose, fructose or galactose. Each animal was given an i.v injection of both prazosin, 0.2 mg/kg and propranolol, 0.5 mg/kg. After forty minutes and basal recording of blood pressure, jejunal blood flow and sample collection for arterial and venous blood glucose, glucose (1.1mg/kg/min) was intravenously infused for twenty minutes. Similar measurements to those used in group 1 were made. Again, another two subgroups (of five dogs per subgroup) were studied using fructose infusion (1.1 mg/kg/min) or galactose infusion (1.1 mg/kg/min) instead of glucose.

Blood glucose was determined by the modified glucose oxidase method (Trinder, 1969). Glucose uptake (mg/min) was calculated as the product of the arterio-venous glucose difference (A -V) and the jejunal blood flow.

Statistical analysis:

All values given are the mean ± S.E.M of the variable measured. Significance was assessed by the Students t-test for two means of independent variables. P values of 0.05 or less were taken as statistically significant

RESULTS

Effects of Glucose, Fructose and Galactose on Blood Glucose Levels.

The effects of the three sugars bood glucose levels are shown in tables 1, 2, and 3. Infusion of glucose, fructose or galactose causes significant increases in blood glucose levels. For instance, the blood glucose level increased from a basal level of 97.4 ± 0.87 mg/gl to 141.2 ± 5.65, 114.2 ± 1.88 and 109.75 ± 1.84 mg/dl following infusion of glucose, fructose and galactose respectively. Again, apart from the three sugars producing different degrees of

hyperglycemia, the maximum level of blood glucose was achieved at different times for the three sugars.

The effecs of adrenergic receptor blockade on the increases in blood glucose and intestinal glucose uptake are shown in tables 1,2 and 3 and figures 1, 2 and 3.

Effects of alpha adrenergic blocker

Prazosin pre-treatment caused significant reduction in glucose or fructose-induced increases in blood glucose levels. Prazosin however did not affect galactose induced hyperglycemia (tables 1 and 2). While prazosin had no effect on glucose or fructose-induced increases in intestinal glucose extraction and uptake, it however completely abolished the galactose-induced intestinal glucose uptake.

Effects of beta adrenergic blocker

Pre-treatment of the dog with propranolol followed by glucose infusion produced significant decreases in arterial blood glucose compared to infusion of glucose alone (Table 1). Pretreatment with propranolol also reduced significantly intestinal glucose uptake in response to glucose infusion. For instance, pretreatment with propranolol caused about 400% reduction in glucose-induced increase intestinal glucose uptake (figure 1). The increase in the intestinal glucose uptake decreased from 670% for untreated dogs to 200% for propranolol-treated dogs (figure 1). Also, propranolol significantly reduced fructose-induced increases in arterial blood glucose. Figure 2 shows the effects of fructose infusion on intestinal glucose uptake in dogs pretreated with propranolol. Propranolol abolished the fructose-induced increase in intestinal glucose uptake (figure 2). Figure 3 shows the effects of galactose on intestinal glucose uptake in untreated and propranolol-treated dogs. Pretreatment of the dog with propranolol also completely abolished the galactose-induced increase in intestinal glucose uptake.

Effects of combined alpha and beta adrenergic blockers

A combination of propranolol and prazosin also caused significant decreases in arterial blood glucose levels (table 1) during and after glucose infusion. The blood glucose was also significantly reduced in the animal pre-treated with the two blockers and infused with fructose. (Table 2). However, a combination of the two adrenergic blockers had no effect on the blood glucose level induced by

Table 1: Effects of intravenous infusion of glucose (Glu) (1.1 mg/kg/min) on arterial glucose concentration (mg/dl) before and after pre-treatment with adrenergic blockers. Pro (Propranolol), Pra (Prazosin) , (*p<0.05)

Treatment	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Glu	97.4 ±0.87	115.8 ±1.32	119.6 ±2.42	126 ±1.70	131.8 ±4.79	134.4 ±5.85	141.2 ±5.65	131.6 ±7.37	132.4 ±6.42	119.8 ±1.71	113.2 ±1.85
Pro+Glu	97.5 ±3.23	103.5 ±2.53*	109.25 ±1.49*	114 ±2.16*	119 ±4.04	117.5 ±4.33	109.25 ±5.65*	106.75 ±4.77*	102.25 ±3.22*	98.5 ±1.55*	95.5 ±1.66*
Pra+Glu	97.25 ±1.11	104.25 ±2.17*	110 ±1.41*	114.25 ±2.17*	122.5 ±2.63	122.5 ±1.04	119.5 ±0.50*	116.25 ±1.03	114.25 ±1.49*	105.25 ±1.89*	101.75 ±1.49*
Pro+pra+Glu	95.5 ±2.10	101.75 ±2.69*	108.0 ±1.41*	112.75 ±1.60*	123.75 ±2.39	123.0 ±1.22	118.75 ±2.39	116.0 ±2.00	103.25 ±1.97*	99.0 ±0.58*	95.75 ±0.75*

Table 2: Effects of intravenous infusion of fructose (Fru) (1.1 mg/kg/min) on arterial glucose concentration (mg/dl) before and after pre-treatment with adrenergic blockers. Pro (Propranolol), Pra (Prazosin) , (*p<0.05)

Treatment	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Fru	97.6 ±1.78	110.6 ±1.25	112 ±2.72	108.8 ±3.01	114.2 ±1.88	103.6 ±3.70	110.8 ±1.28	103.4 ±2.18	109.6 ±1.21	107.4 ±1.78	98.8 ±2.31
Pro+Fru	95.25 ±2.32	97.75 ±3.57*	106 ±1.35	101.75 ±4.44	98.5 ±1.55*	97.25 ±1.49	97 ±0.71*	95.5 ±0.65*	90 ±3.54*	91.75 ±2.02*	94.5 ±0.65
Pra+Fru	99.75 ±2.32	100.75 ±1.11*	99 ±2.16	97 ±3.58*	102.75 ±2.10*	100.75 ±1.49	94.75 ±1.03*	94.5 ±1.32*	92.75 ±0.85*	93.5 ±0.96*	94.75 ±1.03
Pro+pra+Fru	98.25 ±2.78	100.75 ±2.17*	116 ±4.34	104 ±2.48	106.25 ±1.80*	102.5 ±1.76	101 ±0.58*	98.5 ±2.47	100 ±2.04*	100.25 ±1.49*	99.25 ±0.85

Table 3: Effects of intravenous infusion of galactose (Gal) (1.1 mg/kg/min) on arterial glucose concentration (mg/dl) before and after pre-treatment with adrenergic blockers. Pro (Propranolol), Pra (Prazosin) , (*p<0.05)

Treatment	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Gal	96 ±1.47	102.75 ±1.93	106.25 ±1.65	109.75 ±1.84	105.25 ±1.80	102 ±1.63	99 ±1.35	91.75 ±1.18	90.25 ±1.03	86 ±0.71	86 ±1.47
Pro+Gal	96.25 ±1.75	107.75 ±1.11	112.25 ±1.03	114.75 ±2.14	101.5 ±2.53	98.5 ±0.65	92.75 ±3.92	94.25 ±3.79	96.25 ±0.48	96.75 ±0.48	94 ±1.68
Pra+Gal	96.67 ±0.88	107.33 ±0.67	110.33 ±0.33	114 ±1.00	105 ±2.08	101 ±1.00	92 ±1.53	91.33 ±0.33	94.67 ±0.33	94.67 ±1.20	94.67 ±1.33
Pro+pra+Gal	99.5 ±2.90	100.75 ±2.87	108.25 ±2.29	112.5 ±2.63	107.75 ±3.52	99.75 ±0.85	99 ±1.78	98 ±2.48	96 ±0.82	98.75 ±4.21	96.5 ±0.65

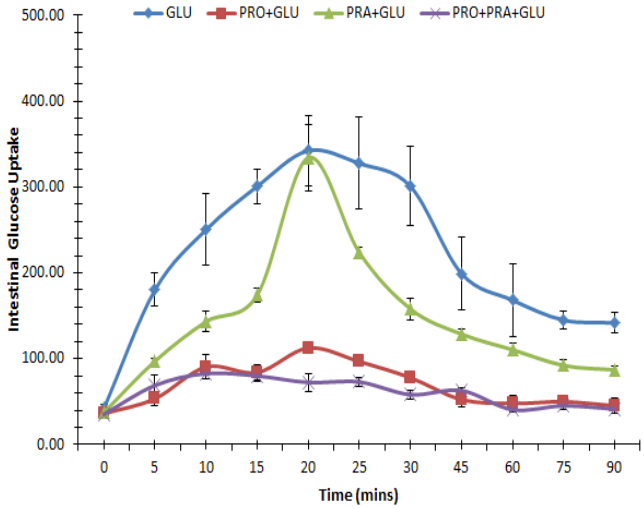

Fig 1: Effects of glucose (GLU) infusion on intestinal glucose uptake before and after pretreatment with prazosin (PRA) and propranolol (PRO).

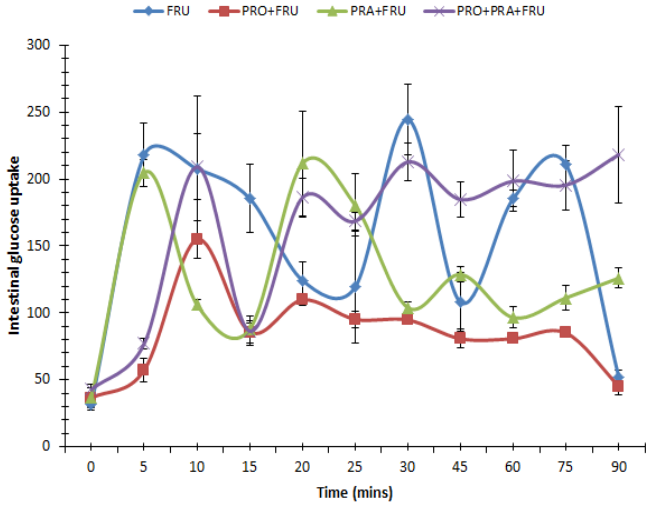

Fig 2: Effects of fructose (FRU)infusion on intestinal glucose uptake before and after pretreatment with prazosin (PRA) and propranolol (PRO).

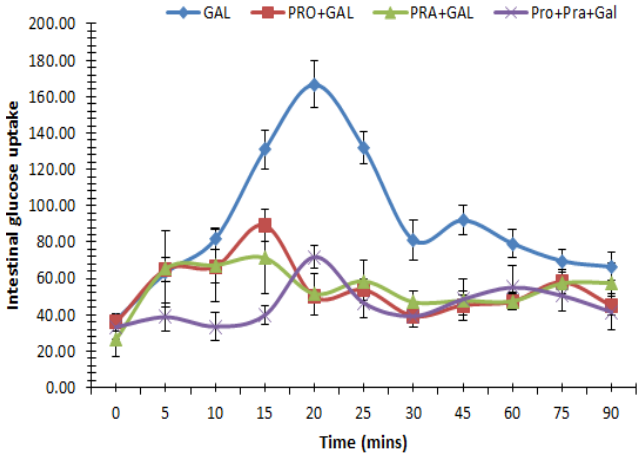

Fig 3 :Effects of galactose (GAL) infusion on intestinal glucose uptake before and after pretreatment with prazosin (PRA) and propranolol (PRO).

galactose. Interestingly, the combined prazosin and propranolol completely abolished the increases in intestinal glucose uptake following infusion of glucose, fructose or galactose. (Figures 1,2 and 3). The latter effect is essentially similar to that of propranolol.

DISCUSSION

The observed increases in blood glucose levels and intestinal glucose uptake following the infusion of fructose, galactose and glucose have been well descibed in a recent report (Salman et al; 2014). The increased intestinal glucose uptake following hyperglycemia induced by glucose, fructose or galactose is also consistent with our recent findings (Salman et al; 2014). The increased glucose uptake by the gut is most probably a metabolic response to the induced hyperglycemia as earlier described in previous findings (Grayson and Oyebola, 1983, Alada and Oyebola, 1996). In other words, the gut responds to the hyperglycemia induced by hexoses such as glucose, fructose or galactose by increasing its glucose uptake.

The most significant observation on the role of adrenergic receptors in the increased intestinal glucose uptake following glucose infusion is the effect of beta adrenergic blocker. Prazosin had no effect on the glucose-induced increase in intestinal glucose uptake. However, propanolol completely abolished the glucose-induced increase in intestinal glucose uptake. These findings are consistent with earlier observation on the effects of alpha and beta adrenergic receptor blockers in similar dog experiments on the intestine (Grayson and Oyebola, 1983; Alada and Oyebola, 1997) and hindlimb (Salahdeen and Alada, 2009). The present study therefore showed that the increase in intestinal glucose uptake in response to the high blood glucose caused by glucose infusion is most probably mediated through beta adrenergic receptors alone.

The significant reduction in fructose-induced increased intestinal glucose uptake by propranolol seems to suggest that the fructose effect on intestinal glucose uptake is also mediated through beta adrenergic receptors. It is to be noted also that propranolol also reduced significantly the arterial blood glucose levels in this study. Therefore, the decrease in intestinal glucose uptake could as well be a consequence of the significant reduction in fructose-induced hyperglycemia. The significant reductions in blood glucose after pretreatment with propranolol are consistent with the reports that propranolol causes significant reduction of blood glucose in man and rat (Allison et al, 1969, Oyebola and Alada, 1993). However, the decrease in blood glucose level in animals pretreated with prazosin followed by infusion of fructose seems to suggest the involvement of alpha adrenergic receptors in fructose induced hyperglycemia. Inspite of the role of alpha adrenergic receptors in fructose-induced hyperglycemia, prazosin had no effect on fructose induced increase in intestinal glucose uptake. That is, frctose induced increase in gut's glucose uptake is most probably due to activation of the beta adrenergic receptors since propranolol abolished the fructose

induced increase in the intestinal glucose uptake. The absence of any effect of prazosin pretreatment on fructose induced increase in intestinal glucose uptake suggests that the fructose effects are not mediated through alpha adrenergic receptors.

The absence of the effects of prazosin or propranolol on the hyperglycemia induced by galactose suggests that both alpha and beta adrenergic receptors are not involved in galactose induced hyperglycemia. Interesting, the two adrenergic receptor blockers reduced considerably galactose-induced increase in the intestinal glucose uptake suggesting that both alpha and beta receptors are involved in galactose-induced increase in intestinal glucose uptake

In conclusion, the present study showed that beta adrenergic receptors are involved in the increased intestinal glucose uptake produced by glucose, fructose and galactose. It also showed that while alpha adrenergic receptors had no role in fructose- and glucose-induced increases in intestinal glucose uptake, they were involved in the increased intestinal glucose uptake caused by galactose.

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In Vitro Antioxidant Properties of Methanolic Leaf Extract of *Vernonia Amygdalina Del*

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Summary: Various methods employed in evaluating antioxidant activities of various samples gives varying results depending on the specificity of the free radical or oxidant used as a reactant. This study investigated the antioxidant /radical scavenging properties of the methanolic extract of *Vernonia amygdalina* (MEVA) leaves and studied the relationship between the assay methods. Antioxidant capacity of MEVA was evaluated by measuring the radical scavenging activity (RSA) of MEVA on 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), nitric oxide (NO) and hydrogen peroxide (HP), hydroxyl radical ($OH^{\bullet}$) scavenging activity (HRSA), lipid peroxidation inhibition activity (LPIA) against 2,2,-azobis(2-amidinopropane) hydrochloride (AAPH) and Trolox Equivalent Antioxidant Capacity (TEAC) of MEVA against 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+}$) radicals as well as the reducing power (RP). Assay methods were subjected to regression analysis and their correlation coefficients calculated. Results were analysed using student's *t*-test and ANOVA. MEVA exhibited highest percentage RSA of 85.8% on HP, followed by $DPPH^{\bullet}$ (29.6%), $OH^{\bullet}$ (26.4%) and least on $NO^{\bullet}$ (21.8%). MEVA inhibited AAPH-induced lipid peroxidation by 30.0% and ABTS-induced radical by 1489% with a marked RP of 0.242±0.01. DPPH correlated excellently with RP (r^2 = 0.86), TEAC (r^2 = 0.94) and HRSA (r^2 = 0.89), the four having good relationship with each other, while LPIA correlated moderately with HP (r^2 = 0.48 and NO (r^2 = 0.34). MEVA exhibited significant free radical scavenging and antioxidant activities. The assay methods correlates very well and could therefore be employed for investigating and understanding antioxidant properties and scavenging activities of plant materials.

Keywords*:* Antioxidants, assay methods, correlation coefficient, free radicals, radical scavenging activity, *Vernonia amygdalina*

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INTRODUCTION

Free radicals and reactive species are widely believed to contribute to the development of several diseases by causing oxidative stress and ultimately oxidative damage (Halliwell, 2007; Ll-Bahr, 2013; Gan and Johnson, 2013; Aruoma *et al*., 2014; Wong and Duce, 2014; Vitetta and Linnane, 2014) which has been implicated in many pathological diseases (Lobo *et al*., 2010; Aprioku, 2013; Bhattacharyya *et al*., 2014; Urrutia *et al*., 2014).

Many years back, Halliwell (2001), alongside with other scientists (Liu *et al*., 2002a; Liu *et al*.,2003) came up with the fact that if oxidative damage could be found to be responsible for the ever increasing incidence of various pathological conditions, then timely actions that could decrease or prevent its occurrence would be therapeutically beneficial. It was then suggested that successful antioxidant treatment should be employed in the delay or prevention of onset of diseases induced by oxidative damage (Halliwell 2002a; 2002b; Galli *et al*., 2002; Steinberg and Witztum, 2002). Since then, knowledge regarding the chemical nature and mechanism of action of antioxidants, especially endogenous antioxidant and their important roles in disease prevention and treatment has been rapidly increasing (Neergheen *et al*., 2006; Halliwell, 2011; 2012; 2013; Choudhari et al., 2014). In view of this, much attention has been focused on the protective biochemical functions of naturally occurring antioxidants in biological systems, and the mechanisms of their actions.

The phenolic compounds, which are widely distributed in plants, were considered to play a very important role as dietary antioxidant component for the prevention of oxidative damage in living systems (Perron *et al*., 2008; Perron and Brumaghim, 2009; Albarracin *et al*., 2012; Aboul-Enein *et al*., 2013; Gao *et al*., 2014). Flavonoids are a large group of polyphenolic compounds abundantly present in

human diet (Gonzalez *et al*., 2011), first identified as plant pigment but later recognised as very potent antioxidant and immunomodulators (Middleton *et al*., 2000; Krifa *et al*., 2013). Flavonoids have been referred to as 'nature's biological modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activities (Yamamoto and Gaynor, 2001) which are attributable to the phenolic hydroxyl groups attached to the flavonoid structure (Divakaran *et al*., 2013; Pereira *et al*., 2013). In recent times, flavonoids have attracted tremendous interest as possible therapeutics against free radical mediated diseases (Middleton *et al*., 2000; Ishizawa, 2011; Grassi *et al*., 2013; Kamel *et al*., 2014; Peng *et al*., 2014; Liang *et al*., 2014).

Vernonia amygdalina (*Astereacea*) is a shrub or small tree of between 1 and 5m in height which grows throughout tropical Africa. The plant commonly known as bitter leaf due to the bitterness of its leaves is well cultivated and is common market merchandise in some African countries like Nigeria, Cameroon, Ethiopia and Zimbabwe. All parts of the plant have been found to be pharmacologically useful. In Nigeria, leaves of the plant are used as a green vegetable or spice in the popular bitter-leaf soup. The leaves could also be macerated and the water extract taken as appetizer or digestive tonic (Singha, 1972; Igile *et al*., 1995; Adesanoye and Farombi, 2010; Momoh *et al*., 2012). Many herbalists and naturopathic Doctors have recommended the aqueous extract for the treatment of ailments like diabetes, dysentery, gastrointestinal problems, nausea and appetite-induced abrosia. The root and the leaves are also used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort (Burkill, 1985; Hamowia and Saffaf, 1994; Ojiako and Nwanjo, 2006; Adesanoye *et al*., 2013; Yedjou *et al*., 2013). Various phytoconstituents have been discovered and isolated from *Vernonia amygdalina* (Farombi and Owoeye, 2011; Ijeh and Ejike, 2011; Toyang and Verpoorte , 2013). This study was designed to evaluate the antioxidant and radical scavenging activities of methanolic extract of *Vernonia amygdalina* (MEVA) *in vitro* and to study the correlation between the methods employed.

MATERIALS AND METHODS

Plant material

The leaves of *Vernonia amygdalina* were obtained from gardens of Forestry Research Institute of Nigeria (FRIN), Ibadan and were authenticated at the herbarium of the Institute. The powdered leaves were packed into soxhlet extractor and defatted with n-hexane. The defatted leaves was extracted in absolute methanol and the methanolic fraction evaporated in water bath at 40 - 50 ^{0}C to obtain a concentrated extract from which stock solutions were prepared.

Reducing power of MEVA

Reducing power of MEVA was determined according to the method of Oyiazu, (1986). Graded amounts of the extract (10-800 µg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50^{o}C for 20 minutes. A portion (2.5 ml) of 10% TCA was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging effect of MEVA on Hydrogen peroxide

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*., (1989). Extracts doses in 4 ml distilled water (20-400µg) were added to hydrogen peroxide solution (0.6 ml). Absorption of hydrogen peroxide was determined 10 minutes later against a blank solution containing extract in phosphate buffer without hydrogen peroxide. Hydrogen peroxide concentration was determined spectrophotometrically from absorbance at 230 nm using the molar absorptivity of $81M^{-1}cm^{-1}$ (Beers and Sizer, 1952).

Determination of the effect of MEVA on DPPH radical

Effect of MEVA on 1,1-dipheny-2-picrylhydrazyl ($DPPH^{\cdot}$) radical was estimated according to the method of Hatano *et al*.,(1988). MEVA (25-500 µg) in 4 ml of distilled water was added to a methanolic solution of DPPH (1 mM, 1 ml). The mixture was shaken and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Catechin (50 µg) was used as standard. The radical scavenging activity (RSA) was calculated as percentage of $DPPH^{\cdot}$ discolouration using the equation: % RSA = 100 x $(1-A_E/A_D)$ where A_E is the absorbance of the solution with extract, and A_D is the absorbance of the $DPPH^{\cdot}$ solution without extract.

Trolox equivalent antioxidant capacity (TEAC) of MEVA

TEAC of MEVA was carried out using an improved 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) decolourization assay (Re *et al*., 1999) as described by Neergheen *et al*., (2006). The $ABTS^+$ radical was generated by a reaction between ABTS (0.5 mM) and 1 mM potassium persulfate each in 100 ml of 0.1 M phosphate buffer. To 3 ml of the $ABTS^+$ solution, 0.5 ml of the extract was added and the decay in absorbance was followed for 6 minutes at 734 nm. Trolox was used as a

reference standard and TEAC values were expressed as µmol trolox equivalent.

Effect of MEVA on 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH)-induced lipid peroxidation

The effect of MEVA on AAPH-induced lipid peroxidation was carried out according to the method described by Neergheen *et al*., (2006). An aliquot of 200 µl of post-mitochondrial fraction (PMF) of liver homogenate was diluted in 0.1 M potassium phosphate buffer, pH 7.5 (1 in 10 dilution). Then, 400 µl of extract (100-1000 µg) was added followed by 200 µl of AAPH (20 mM) to initiate peroxidation. The mixture was incubated at 37^{o}C for 1 hour with the solution gently shaken at 10 minutes interval. After incubation, 1.6 ml TCA-TBA-HCl stock solution (15% w/v TCA, 0.375% w/v TBA, 0.25 N HCl) was added to the solution and heated in a boiling water bath for 15 minutes. After cooling, the precipitate was removed by centrifugation and the absorbance of the resulting supernatant measured at 532 nm. Results were expressed as percentage inhibition of peroxidation with catechin used as standard.

Deoxyribose assay – hydroxyl radical scavenging activity of MEVA

The hydroxyl radical ($OH^{\cdot}$) scavenging potential of MEVA was determined using the deoxyribose assay (Halliwell *et al*., 1987; Aruoma, 1994a; 1994b) as described by Neergheen *et al*., 2006. About 200-1000 µg of extract in 100 µl of distilled water was added to a solution containing 200 µl KH_2PO_4 – KOH (100 mM), 200 µl deoxyribose (15 mM), 200 µl $FeCl_3$ (500 µM) and 100 µl EDTA (1 mM) in a test tube and allowed to mix. Then, 100 µl H_2O_2 (10 mM) and 100 µl ascorbic acid (1 mM) were added to initiate the reaction. The reaction mixture was incubated at 37^{o}C for 1 hour after which 1 ml of 1% $^{w}/_{v}$ TBA was added to each mixture followed by the addition of 1ml of 2.8% w/v TCA. The solution was heated in a water bath at 80^{o}C for 20 minutes to develop the pink coloured MDA-$(TBA)_2$ adduct. After cooling, the solution was centrifuged and the absorbance of the supernatant measured at 532 nm against distilled water as blank. Results were expressed as the percentage inhibition of deoxyribose degradation. % inhibition = (Abs control – Abs test/ Abs control) ×100.

Scavenging of nitric oxide radical by MEVA

The scavenging effect of MEVA on nitric oxide ($NO^{\cdot}$) radical was measured according to the method of Marcocci *et al*., 1994. About 10-400 µg of MEVA was added in the test tubes to 1 ml of sodium nitroprusside solution (25 mM) and the tubes incubated at 37^{o}C for 2 hours. An aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank with catechin (50 µg) used as standard. Results were expressed as percentage radical scavenging activity (%RSA) = (1 – ΔAbs of sample/ΔAbs of control) x 100.

RESULTS

The antioxidant ability of methanolic extract of *Vernonia amygdalina* (MEVA) to inhibit, scavenge and quench free radicals and reactive oxygen species or ameliorate their effects was examined in this study. Effect of MEVA on oxidative stress biomarkers was investigated using different method.

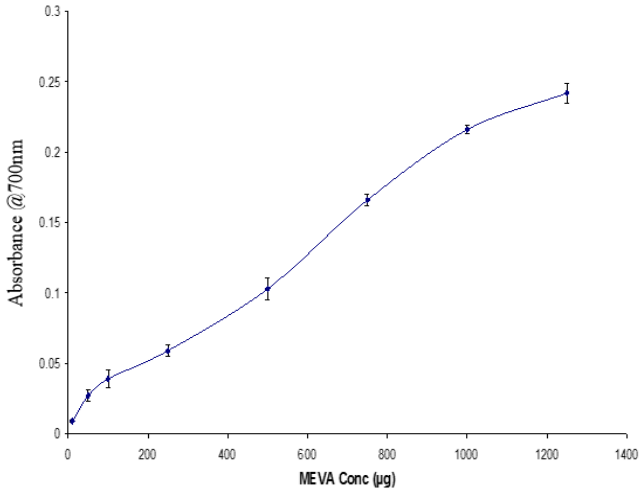

Figure 1: Reducing power of methanolic extract of *Vernonia amygdalina* (MEVA) using the Fe^{3+}/ferricyanide method. Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates.*p<0.05when compared with Control.

Table 1: Radical Scavenging activity (RSA) of methanolic extract of *Vernonia amygdalina* (MEVA) on $DPPH^{\cdot}$ radical

Concentration (µg)	Absorbance	% RSA
Control	1.699± 0.001	-
MEVA 40	1.371±0.060*	19.3
100	1.299±0.004*	23.5
250	1.284±0.016*	24.4
500	1.263±0.003*	25.7
750	1.241±0.032*	27.0
1000	1.196±0.037*	29.6
1500	1.206±0.018*	29.0
2000	1.212±0.013*	28.7
Catechin 50	1.216±0.029*	25.8
100	1.246±0.006*	26.7

Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates. *p<0.05 when compared with control.

The reducing power (RP) of MEVA is shown in Figure 1. MEVA showed significant (p<0.05) reducing power as indicated by the increase in absorbance value from 0.009±0.001 – 0.242±0.007 at

10 – 1250 µg. Table 1 shows the radical scavenging activity (RSA) of MEVA against DPPH$^{\bullet}$ radical exhibiting maximum RSA of 29.6% on DPPH$^{\bullet}$ radical ($p<0.05$) at 1000 µg. Possible inhibitory effect of MEVA on hydrogen peroxide (HP) is displayed in Table 2. MEVA showed highest percentage inhibition of 85.8% on HP at 20 µg (0.102±0.007) thereafter reducing to 24.2% (0.545± 0.021) at 500 µg. At 50 µg, MEVA (84.8%) compares very well with catechin (83.9%), showing similar scavenging activity. The antioxidant capacity of MEVA against ABTS^{+} radical was investigated in the TEAC (trolox equivalent antioxidant capacity) system as presented in Figure 2.

Table 2: Radical scavenging activity (RSA) of methanolic extract of *Vernonia amygdalina* (MEVA) on Hydrogen peroxides.

Concentration (µg)	Absorbance	% Inhibition
Control	0.719±0.007	-
MEVA 20	0.102±0.007*	85.8
50	0.109±0.008*	84.8
100	0.154±0.003*	78.6
200	0.237±0.006*	67.0
300	0.330±0.006*	54.1
400	0.447±0.004*	37.8
500	0.545±0.021*	24.2
Catechin 50	0.116±0.005*	83.9
100	0.161±0.006*	77.6

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

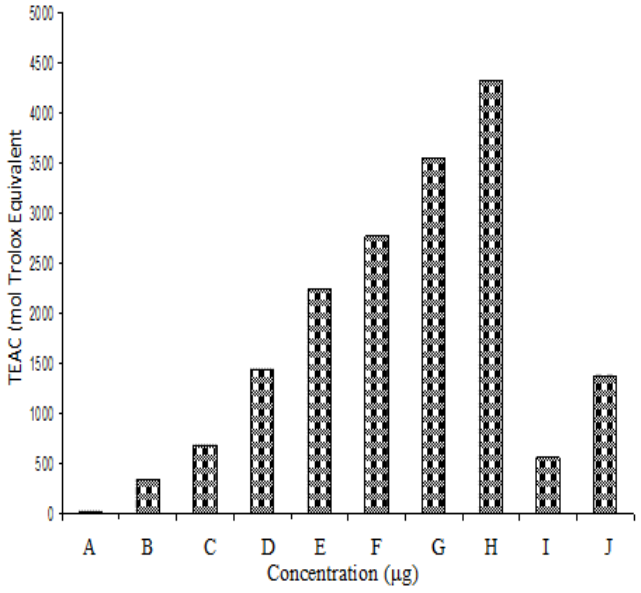

Figure 2: Trolox equivalent antioxidant capacity (TEAC) of methanolic extract of *Vernonia amygdalina* (MEVA). Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates. A=Control, B=20µg MEVA, C=50µg MEVA, D=100µg MEVA, E=200µg MEVA, F=400µg MEVA, G=600µg MEVA, H=1000µg MEVA, I= 50µg catechin, J=100µg catechin.

Table 3: Scavenging effect of methanolic extract of *Vernonia amygdalina* (MEVA) on AAPH-induced lipid peroxidation

Concentration (µg)	Absorbance	% Inhibition
Control	0.087±0.004	-
MEVA 50	0.078± 0.004	10.3
100	0.062±0.003*	28.7
200	0.061±0.0005*	30.0
400	0.073± 0.0001*	16.1
800	0.076±0.001*	12.6
1000	0.081±0.001	6.9
Catechin 50	0.078±0.004	10.3
100	0.058±0.004*	33.3

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Table 4: Potential of methanolic extract of *Vernonia amygdalina* (MEVA) to inhibit deoxyribose degradation by Hydroxyl radical

Concentration (µg)	Absorbance	% Inhibition
Control	0.738±0.006	-
MEVA 50	0.607±0.023*	17.8
100	0.598±0.017*	19.0
200	0.562±0.028*	23.8
400	0.559±0.008*	24.3
600	0.543±0.021*	26.4
800	0.545±0.008*	26.2
1000	0.553±0.008*	25.1
Catechin 50	0.665± 0.015*	9.9
100	0.652±0.002*	11.7

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Table 5: Scavenging effect of methanolic extract of *Vernonia amygdalina* (MEVA) on Nitric Oxide radical

Concentration (µg)	Absorbance	% Inhibition
Control	0.229±0.01	-
MEVA 20	0.179± 0.003*	21.8
50	0.191±0.006*	16.9
100	0.211±0.002*	7.9
200	0.251± 0.002*	-9.6
300	0.287±0.0003*	-25.3
400	0.334±0.007*	-45.9
Catechin 50	0.192±0.001*	16.2
100	0.197±0.001*	14.0

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Likewise, MEVA was able to significantly ($p<0.05$) inhibit AAPH-induced lipid peroxidation by 28.7%, 30.0%, 16.1% and 12.6% at 100, 200, 400 and 800 µg respectively when compared with control (Table 3). Catechin showed greater ability at 100 µg by 33.3% when compared with MEVA (28.7%) at the same concentration. MEVA significantly ($p<0.05$) inhibited OH$^{\bullet}$-induced deoxyribose degradation from 50 – 1000 µg with the highest potency at 600 µg by 26.4% when compared with control as shown in Table 4. Similarly, MEVA exhibited scavenging effect on nitric oxide radical (NO$^{\bullet}$) but at relatively low concentration (Table 5).

Table 6: Correlation Coefficient between Assay methods

Assay methods	**Correlation coefficients (r^2)**
DPPH/RP	0.86
DPPH/LPIA	-0.25
DPPH/TEAC	0.94
DPPH/HP	-0.89
DPPH/HRSA	0.89
DPPH/NO	-0.93
RP/LPIA	-0.57
RP/TEAC	0.97
RP/HP	-0.99
RP/HRSA	0.71
RP/NO	-0.98
LPIA/TEAC	-0.50
LPIA/HP	0.63
LPIA/HRSA	-0.28
LPIA/NO	0.59
TEAC/HP	-0.98
TEAC/HRSA	0.85
TEAC/NO	-0.98
HP/HRSA	-0.76
HP/NO	0.99
HRSA/NO	-0.88

- Negative correlation coefficients

Table 7: Summary of the relationships between Assay methods

DPPH:	RP, TEAC, HRSA
RP:	DPPH, TEAC, HRSA
TEAC:	DPPH, RP, HRSA
HRSA:	DPPH, RP, TEAC
LPIA:	HP, NO
HP:	NO, LPIA
NO:	LPIA, HP

Very strong linear relationships (R^2) were observed between DPPH, RP, TEAC and HRSA while medium to low relationship was observed between LPIA, NO and HP. The level of correlation coefficients between the assays methods are shown in Table 6 while Table 7 summarised the relationship between the various methods.

DISCUSSION

Phytochemicals are diverse and complex in nature. As such, the antioxidant activities of plant extracts cannot be evaluated by only a single method. Different antioxidant components are resident in the antioxidant defence system of the body and the antioxidant capacity of these antioxidant components depends to a great extent on which free radical or oxidants are produced in the system (Choi *et al*., 2002; Akinmoladun *et al*., 2010; Jan *et al*., 2013; Tenore and Ciampaglia, 2013). Therefore, various methods used in evaluating the antioxidant activity of various samples can give varying results depending on the specificity of the free radical used as a reactant (Frankel and Meyer, 2001; Prakash, 2001; Aruoma, 2003; Akinmoladun *et al*., 2010; Patel *et al*., 2011; Ramasarma, 2012; Shabbir *et al*., 2013).

In the present study, MEVA was able to reduce the Fe^{3+}/ferricyanide complex to the ferrous form, Fe^{2+}, which was measured as the formation of Perl's Prussian blue colour at 700nm (Chung *et al*., 2002). MEVA at 250 µg (0.059±0.004) when compared with the reference antioxidant, catechin at 50 µg (0.051±0.003) indicates that the reducing power of MEVA is less than that of catechin, but nevertheless potent enough to function as a good electron and hydrogen-atom donor which can react with free radicals to convert them to more stable products and terminate radical chain reaction. Several studies have also reported the reducing power activity of several plants extracts (Rice-Evans *et al*., 1996; Amarowicz *et al*., 2004; Perez-Perez *et al*., 2006; Manian *et al*., 2008; Sen *et al*., 2013; Rahman *et al*., 2014), and this has been linked to the polyphenolic constituents of these extracts. The assay method used for measuring RP correlates excellently with other methods employed in this study, especially, DPPH, TEAC and HRSA at correlation coefficients (r^2) of 0.86, 0.97 and 0.71 respectively and as such is an excellent method for measuring the reductive ability of antioxidants. Amarowicz *et al*., (2004) in their findings also confirmed that there is a good relationship between RP and DPPH (Sen *et al*., 2013; Das *et al*., 2014).

DPPH radical has been used extensively as a free radical to test the reductive ability of extracts or compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods (Porto *et al*., 2000; Manian *et al*., 2008). Antioxidants react with $DPPH^{\bullet}$ by providing electron or hydrogen atom, thus reducing it to 1,1-diphenyl-2-hydrazine (DPPH-H) or a substituted analogous hydrazine. The deep violet colour of DPPH at maximum absorption of 517 nm is changed to light yellow, colourless or bleached product, resulting in decrease in absorption (Miliauskas *et al*., 2004; Koksal *et al*., 2009). MEVA exhibiting significant scavenging effect on DPPH radical between 40 – 1000 µg (19.3% - 29.6%) compares well with catechin though showing less scavenging activity. Likewise, the assay method of DPPH scavenging activity correlates excellently with RP, TEAC and HRSA with correlation coefficients (r^2) of 0.86, 0.94 and 0.89 respectively. The scavenging effect of plant extracts on DPPH has been shown to be related to the phenolic concentration of the extracts (Manian *et al*., 2008; Akinmoladun *et al*., 2010; Sen *et al*., 2013; Das *et al*., 2014), which is believed to contribute to their electron transfer / hydrogen donating ability. It could therefore be suggested that MEVA contains flavonoids with hydroxyl groups that could stabilize free radicals or scavenge their activities and that the method employed is reliable.

The activity of hydrogen peroxide (HP) as an active oxygen species has been reported to come mainly from its potential to produce the highly reactive hydroxyl radical through the fenton reaction: $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\bullet}$ (Namiki, 1990; Farombi *et al*., 2002; Koksal *et al*., 2009; Jeong *et al*., 2010; Ogasawara *et al*., 2014). Therefore, inhibition of HP formation will prevent further generation of radicals. In the present study, MEVA showed highest percentage inhibition of 85.8% on HP at 20 µg (0.102±0.007) thereafter reducing to 24.2% (0.545± 0.021) at 500 µg. At 50 µg, MEVA (84.8%) compares very well with catechin (83.9%), showing similar scavenging activity. The regression analysis of HP versus other methods in the study revealed a very good relationship between HP scavenging activity and nitric oxide scavenging activity (NO) with a correlation coefficient of 0.99, and a moderate relationship with lipid peroxidation inhibition activity with correlation coefficient of 0.35. Although HP on its own is not very reactive, but it could still be toxic both *in vitro* and *in vivo*; it could induce cell death *in vitro* and attack many cellular energy-producing systems *in vivo*. For instance, it has been reported that HP could deactivate the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (Hyslop *et al*., 1998; Koksal *et al*., 2009; Song *et al*., 2014).

$ABTS^+$ radical was generated by oxidation of ABTS with potassium persulfate (Re *et al*., 1999). MEVA increased TEAC values in a dose dependant manner from 50–600 µg with 339.4±155.3 – 4320.0±33.6 trolox equivalent and shows almost equal potency with catechin at 50 µg. $ABTS^+$, a more reactive radical (more reactive than $DPPH^{\bullet}$) is stabilized by antioxidants that can supply electrons, unlike H atom in $DPPH^{\bullet}$. MEVA was thus able to scavenge $ABTS^+$ radical-induced peroxodisulfate formation by electron transfer. The regression analysis for the assay methods employed in this study reveals that measurement of $ABTS^+$ scavenging activity as Trolox equivalent antioxidant capacity (TEAC) compares very well with other methods in showing strong linear correlation with DPPH, RP and HRSA with correlation coefficients (r^2) of 0.94, 0.97 and 0.85 respectively. This indicates that TEAC is a reliable method for measuring the total antioxidant activities of substances as reported by previous works (Koksal *et al*., 2009; Piljac-Zegarac *et al*., 2009; Chohan *et al*., 2012). Likewise, the report of Sasikumar *et al*., (2010) supported the relationship between TEAC and DPPH.

Lipid peroxidation is one of the consequent actions of ROS and free radicals. In this study, lipid peroxidation was induced with AAPH, a peroxy radical initiator. Thermal decomposition of AAPH produces peroxy radical which can attack polyunsaturated lipids initiating lipid peroxidation (Neergheen *et al*., 2006). MEVA was able to significantly ($p<0.05$) inhibit AAPH-induced lipid peroxidation by 28.7%, 30.0%, 16.1% and 12.6% at 100, 200, 400 and 800 µg respectively when compared with control. Catechin showed greater ability at 100 µg by 33.3% when compared with MEVA (28.7%) at the same concentration.

The decreasing lipid peroxidation product measured as thiobarbituric reactive substances (TBARS) showed the ability of MEVA to inhibit AAPH-induced lipid peroxidation by 10.3% – 30.0%. LPIA (Lipid peroxidation inhibitory activity of MEVA) correlates fairly well with HP and NO; the other methods employed in this study, with correlation coefficients (r^2) of 0.63 and 0.59 respectively and as such, LPIA is another reliable method of measuring the scavenging and inhibitory ability of antioxidants. In the report of Akinmoladun *et al*., (2010), very low to fair correlations were observed between LPIA and other methods, which is similar to what was observed in this study.

Furthermore, the antioxidant activity of MEVA was demonstrated against hydroxyl radical ($OH^{\bullet}$). $OH^{\bullet}$ has been reported to be the most reactive radical known; it attacks and damages almost every molecule it makes contact with (Halliwell, 1989; Aruoma, 1999; Manian *et al*., 2008; Ramasarma, 2012). $OH^{\bullet}$ rapidly reacts with any molecule, forming another radical species, thereby giving rise to chain peroxide formation. The deoxyribose method employed in this study is a simple assay to determine the rate constants of hydroxyl radical formation as reported by Halliwell *et al*., (1987). $OH^{\bullet}$ radical is generated when the mixture of $FeCl_3$-EDTA, H_2O_2 and ascorbate is incubated with deoxyribose in phosphate buffer (pH 7.4). $OH^{\bullet}$ attack on deoxyribose results ultimately in chain peroxidation which could be measured as TBARS. MEVA exhibited 17.8 – 26.4% inhibition of deoxyribose oxidation by $OH^{\bullet}$. The ability of MEVA to inhibit $OH^{\bullet}$ by preventing the oxidation of deoxyribose in the current *in vitro* model could be related to prevention of propagation of the process of lipid peroxidation *in vivo* as has been shown in our studies with CCl_4 (Adesanoye and Farombi, 2010). In this vein, Farombi *et al*., (2002) reported that the overall antioxidant effect of flavonoids on lipid peroxidation is the result of scavenging hydroxyl radicals and superoxide anions at the stage of initiation and termination of peroxyl radicals according to Hussain *et al*., (1987). In addition, MEVA showed better inhibition potential of 17.8% against 9.9% for catechin at 50 µg. The regression analysis in this study revealed a very high relationship between hydroxyl radical scavenging activity (HRSA) and other methods which is in line with some previous works (Perez-Perez *et al*., 2006; Sazikumar *et al*., 2010). HRSA correlated excellently

with DPPH, RP and TEAC, with correlation coefficients (r^2) of 0.89, 0.71 and 0.85 respectively. The relationship between these four methods is excellent as has been observed and reported by various scientists (Halliwell, 1989; Amarowicz *et al*., 2004; Manian *et al*., 2008; Piljac-Zegarac *et al*., 2009; Akinmoladun *et al*., 2010).

Similarly, MEVA exhibited scavenging effect on nitric oxide radical ($NO^{\bullet}$) but at relatively low concentration. $NO^{\bullet}$ shows dual function as both an antioxidant and pro-oxidant depending on the relative ratios of the reactants (Aruoma, 1996; Groß *et al*., 2013; Meenakshi and Agarwal, 2013). Antioxidant effects of $NO^{\bullet}$ occurs when $NO^{\bullet}$ reacts with alkoxy and peroxyl radical intermediates during lipid peroxidation thereby stabilizing the inhibition of LDL oxidation while the pro-oxidant reaction occurs when $NO^{\bullet}$ reacts with $O_2^{\bullet}$ to yield peroxynitrite ($ONOO^{\bullet}$) (Zielonka et al., 2010). This dual effect could explain why the scavenging effect on $NO^{\bullet}$ decreased with increasing concentration of the extract and became pro-oxidant at concentrations greater than 100 µg. MEVA exhibited similar scavenging effect with catechin. At 50 µg, MEVA elicited 16.9% inhibition while catechin showed 16.2% inhibition potential. The ability of MEVA to scavenge $NO^{\bullet}$ and especially its deleterious metabolite, ($ONOO^{\bullet}$) will be highly beneficial in biological system as $ONOO^{\bullet}$ and some other $NO^{\bullet}$ metabolites have been implicated in various pathological conditions such as malaria, cardiovascular diseases, inflammation, cancer and diabetes (Aruoma, 1996; Groß et al., 2013; Meenakshi and Agarwal, 2013). NO correlated with LPIA and HP with correlation coefficients (r^2) of 0.59 and 0.99 respectively.

CONCLUSION

In conclusion, this study demonstrated that MEVA is a potent antioxidant and scavenger of reactive species *in vitro*. The observation concerning the diversity and complexity of the phytochemicals in plant extracts with different mechanisms of reaction for specific antioxidant species applies very well to the present study. The antioxidant capacity observed by all the methods assayed in this work appears to be reliable for measuring antioxidant and scavenging capacity of substances. From various experimental results (Dasgupta and De, 2004; Perez-Perez *et al*., 2006; Akinmoladun *et al*., 2010; Rao *et al*., 2010; Sasikumar *et al*., 2010; Patel *et al*., 2011; Ramasarma, 2012; Shabbir *et al*., 2013), a linear correlation with high correlation coefficients has been observed between total phenolic/flavonoid content and various antioxidant and free radical scavenging activities of various plants indicating that the various activities are based on the flavonoid and phenolic contents of the plant materials. It could therefore be inferred that the activities of MEVA in this study is due to its phenolic content, especially the flavonoids. Therefore, MEVA could be recommended as a potential antioxidant in ameliorating oxidative and free radical-induced pathologies.

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In Vitro Antioxidant Properties of Methanolic Leaf Extract of *Vernonia Amygdalina Del*

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Summary: Various methods employed in evaluating antioxidant activities of various samples gives varying results depending on the specificity of the free radical or oxidant used as a reactant. This study investigated the antioxidant /radical scavenging properties of the methanolic extract of *Vernonia amygdalina* (MEVA) leaves and studied the relationship between the assay methods. Antioxidant capacity of MEVA was evaluated by measuring the radical scavenging activity (RSA) of MEVA on 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), nitric oxide (NO) and hydrogen peroxide (HP), hydroxyl radical ($OH^{\bullet}$) scavenging activity (HRSA), lipid peroxidation inhibition activity (LPIA) against 2,2,-azobis(2-amidinopropane) hydrochloride (AAPH) and Trolox Equivalent Antioxidant Capacity (TEAC) of MEVA against 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+}$) radicals as well as the reducing power (RP). Assay methods were subjected to regression analysis and their correlation coefficients calculated. Results were analysed using student's *t*-test and ANOVA. MEVA exhibited highest percentage RSA of 85.8% on HP, followed by $DPPH^{\bullet}$ (29.6%), $OH^{\bullet}$ (26.4%) and least on $NO^{\bullet}$ (21.8%). MEVA inhibited AAPH-induced lipid peroxidation by 30.0% and ABTS-induced radical by 1489% with a marked RP of 0.242±0.01. DPPH correlated excellently with RP (r^2 = 0.86), TEAC (r^2 = 0.94) and HRSA (r^2 = 0.89), the four having good relationship with each other, while LPIA correlated moderately with HP (r^2 = 0.48 and NO (r^2 = 0.34). MEVA exhibited significant free radical scavenging and antioxidant activities. The assay methods correlates very well and could therefore be employed for investigating and understanding antioxidant properties and scavenging activities of plant materials.

Keywords*:* Antioxidants, assay methods, correlation coefficient, free radicals, radical scavenging activity, *Vernonia amygdalina*

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INTRODUCTION

Free radicals and reactive species are widely believed to contribute to the development of several diseases by causing oxidative stress and ultimately oxidative damage (Halliwell, 2007; Ll-Bahr, 2013; Gan and Johnson, 2013; Aruoma *et al*., 2014; Wong and Duce, 2014; Vitetta and Linnane, 2014) which has been implicated in many pathological diseases (Lobo *et al*., 2010; Aprioku, 2013; Bhattacharyya *et al*., 2014; Urrutia *et al*., 2014).

Many years back, Halliwell (2001), alongside with other scientists (Liu *et al*., 2002a; Liu *et al*.,2003) came up with the fact that if oxidative damage could be found to be responsible for the ever increasing incidence of various pathological conditions, then timely actions that could decrease or prevent its occurrence would be therapeutically beneficial. It was then suggested that successful antioxidant treatment should be employed in the delay or prevention of onset of diseases induced by oxidative damage (Halliwell 2002a; 2002b; Galli *et al*., 2002; Steinberg and Witztum, 2002). Since then, knowledge regarding the chemical nature and mechanism of action of antioxidants, especially endogenous antioxidant and their important roles in disease prevention and treatment has been rapidly increasing (Neergheen *et al*., 2006; Halliwell, 2011; 2012; 2013; Choudhari et al., 2014). In view of this, much attention has been focused on the protective biochemical functions of naturally occurring antioxidants in biological systems, and the mechanisms of their actions.

The phenolic compounds, which are widely distributed in plants, were considered to play a very important role as dietary antioxidant component for the prevention of oxidative damage in living systems (Perron *et al*., 2008; Perron and Brumaghim, 2009; Albarracin *et al*., 2012; Aboul-Enein *et al*., 2013; Gao *et al*., 2014). Flavonoids are a large group of polyphenolic compounds abundantly present in

human diet (Gonzalez *et al*., 2011), first identified as plant pigment but later recognised as very potent antioxidant and immunomodulators (Middleton *et al*., 2000; Krifa *et al*., 2013). Flavonoids have been referred to as 'nature's biological modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activities (Yamamoto and Gaynor, 2001) which are attributable to the phenolic hydroxyl groups attached to the flavonoid structure (Divakaran *et al*., 2013; Pereira *et al*., 2013). In recent times, flavonoids have attracted tremendous interest as possible therapeutics against free radical mediated diseases (Middleton *et al*., 2000; Ishizawa, 2011; Grassi *et al*., 2013; Kamel *et al*., 2014; Peng *et al*., 2014; Liang *et al*., 2014).

Vernonia amygdalina (*Astereacea*) is a shrub or small tree of between 1 and 5m in height which grows throughout tropical Africa. The plant commonly known as bitter leaf due to the bitterness of its leaves is well cultivated and is common market merchandise in some African countries like Nigeria, Cameroon, Ethiopia and Zimbabwe. All parts of the plant have been found to be pharmacologically useful. In Nigeria, leaves of the plant are used as a green vegetable or spice in the popular bitter-leaf soup. The leaves could also be macerated and the water extract taken as appetizer or digestive tonic (Singha, 1972; Igile *et al*., 1995; Adesanoye and Farombi, 2010; Momoh *et al*., 2012). Many herbalists and naturopathic Doctors have recommended the aqueous extract for the treatment of ailments like diabetes, dysentery, gastrointestinal problems, nausea and appetite-induced abrosia. The root and the leaves are also used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort (Burkill, 1985; Hamowia and Saffaf, 1994; Ojiako and Nwanjo, 2006; Adesanoye *et al*., 2013; Yedjou *et al*., 2013). Various phytoconstituents have been discovered and isolated from *Vernonia amygdalina* (Farombi and Owoeye, 2011; Ijeh and Ejike, 2011; Toyang and Verpoorte , 2013). This study was designed to evaluate the antioxidant and radical scavenging activities of methanolic extract of *Vernonia amygdalina* (MEVA) *in vitro* and to study the correlation between the methods employed.

MATERIALS AND METHODS

Plant material

The leaves of *Vernonia amygdalina* were obtained from gardens of Forestry Research Institute of Nigeria (FRIN), Ibadan and were authenticated at the herbarium of the Institute. The powdered leaves were packed into soxhlet extractor and defatted with n-hexane. The defatted leaves was extracted in absolute methanol and the methanolic fraction evaporated in water bath at 40 - 50 ^{0}C to obtain a concentrated extract from which stock solutions were prepared.

Reducing power of MEVA

Reducing power of MEVA was determined according to the method of Oyiazu, (1986). Graded amounts of the extract (10-800 µg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50^{o}C for 20 minutes. A portion (2.5 ml) of 10% TCA was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging effect of MEVA on Hydrogen peroxide

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*., (1989). Extracts doses in 4 ml distilled water (20-400µg) were added to hydrogen peroxide solution (0.6 ml). Absorption of hydrogen peroxide was determined 10 minutes later against a blank solution containing extract in phosphate buffer without hydrogen peroxide. Hydrogen peroxide concentration was determined spectrophotometrically from absorbance at 230 nm using the molar absorptivity of $81M^{-1}cm^{-1}$ (Beers and Sizer, 1952).

Determination of the effect of MEVA on DPPH radical

Effect of MEVA on 1,1-dipheny-2-picrylhydrazyl ($DPPH^{\cdot}$) radical was estimated according to the method of Hatano *et al*.,(1988). MEVA (25-500 µg) in 4 ml of distilled water was added to a methanolic solution of DPPH (1 mM, 1 ml). The mixture was shaken and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Catechin (50 µg) was used as standard. The radical scavenging activity (RSA) was calculated as percentage of $DPPH^{\cdot}$ discolouration using the equation: % RSA = 100 x $(1-A_E/A_D)$ where A_E is the absorbance of the solution with extract, and A_D is the absorbance of the $DPPH^{\cdot}$ solution without extract.

Trolox equivalent antioxidant capacity (TEAC) of MEVA

TEAC of MEVA was carried out using an improved 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) decolourization assay (Re *et al*., 1999) as described by Neergheen *et al*., (2006). The $ABTS^+$ radical was generated by a reaction between ABTS (0.5 mM) and 1 mM potassium persulfate each in 100 ml of 0.1 M phosphate buffer. To 3 ml of the $ABTS^+$ solution, 0.5 ml of the extract was added and the decay in absorbance was followed for 6 minutes at 734 nm. Trolox was used as a

reference standard and TEAC values were expressed as µmol trolox equivalent.

Effect of MEVA on 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH)-induced lipid peroxidation

The effect of MEVA on AAPH-induced lipid peroxidation was carried out according to the method described by Neergheen *et al*., (2006). An aliquot of 200 µl of post-mitochondrial fraction (PMF) of liver homogenate was diluted in 0.1 M potassium phosphate buffer, pH 7.5 (1 in 10 dilution). Then, 400 µl of extract (100-1000 µg) was added followed by 200 µl of AAPH (20 mM) to initiate peroxidation. The mixture was incubated at 37^{o}C for 1 hour with the solution gently shaken at 10 minutes interval. After incubation, 1.6 ml TCA-TBA-HCl stock solution (15% w/v TCA, 0.375% w/v TBA, 0.25 N HCl) was added to the solution and heated in a boiling water bath for 15 minutes. After cooling, the precipitate was removed by centrifugation and the absorbance of the resulting supernatant measured at 532 nm. Results were expressed as percentage inhibition of peroxidation with catechin used as standard.

Deoxyribose assay – hydroxyl radical scavenging activity of MEVA

The hydroxyl radical ($OH^{\cdot}$) scavenging potential of MEVA was determined using the deoxyribose assay (Halliwell *et al*., 1987; Aruoma, 1994a; 1994b) as described by Neergheen *et al*., 2006. About 200-1000 µg of extract in 100 µl of distilled water was added to a solution containing 200 µl KH_2PO_4 – KOH (100 mM), 200 µl deoxyribose (15 mM), 200 µl $FeCl_3$ (500 µM) and 100 µl EDTA (1 mM) in a test tube and allowed to mix. Then, 100 µl H_2O_2 (10 mM) and 100 µl ascorbic acid (1 mM) were added to initiate the reaction. The reaction mixture was incubated at 37^{o}C for 1 hour after which 1 ml of 1% $^{w}/_{v}$ TBA was added to each mixture followed by the addition of 1ml of 2.8% w/v TCA. The solution was heated in a water bath at 80^{o}C for 20 minutes to develop the pink coloured MDA-$(TBA)_2$ adduct. After cooling, the solution was centrifuged and the absorbance of the supernatant measured at 532 nm against distilled water as blank. Results were expressed as the percentage inhibition of deoxyribose degradation. % inhibition = (Abs control – Abs test/ Abs control) ×100.

Scavenging of nitric oxide radical by MEVA

The scavenging effect of MEVA on nitric oxide ($NO^{\cdot}$) radical was measured according to the method of Marcocci *et al*., 1994. About 10-400 µg of MEVA was added in the test tubes to 1 ml of sodium nitroprusside solution (25 mM) and the tubes incubated at 37^{o}C for 2 hours. An aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank with catechin (50 µg) used as standard. Results were expressed as percentage radical scavenging activity (%RSA) = (1 – ΔAbs of sample/ΔAbs of control) x 100.

RESULTS

The antioxidant ability of methanolic extract of *Vernonia amygdalina* (MEVA) to inhibit, scavenge and quench free radicals and reactive oxygen species or ameliorate their effects was examined in this study. Effect of MEVA on oxidative stress biomarkers was investigated using different method.

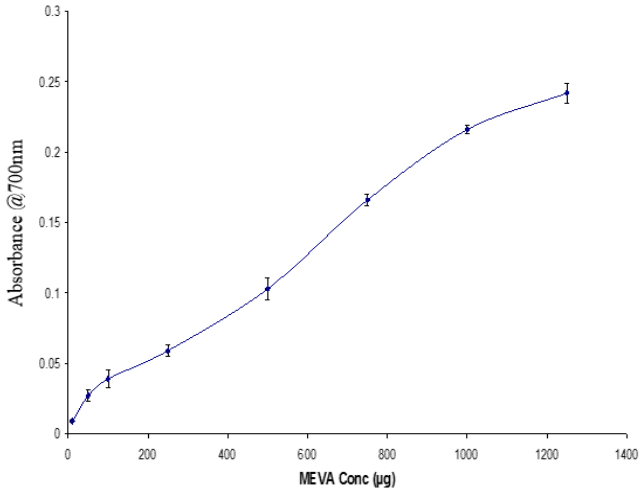

Figure 1: Reducing power of methanolic extract of *Vernonia amygdalina* (MEVA) using the Fe^{3+}/ferricyanide method. Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates.*p<0.05when compared with Control.

Table 1: Radical Scavenging activity (RSA) of methanolic extract of *Vernonia amygdalina* (MEVA) on $DPPH^{\cdot}$ radical

Concentration (µg)	Absorbance	% RSA
Control	1.699± 0.001	-
MEVA 40	1.371±0.060*	19.3
100	1.299±0.004*	23.5
250	1.284±0.016*	24.4
500	1.263±0.003*	25.7
750	1.241±0.032*	27.0
1000	1.196±0.037*	29.6
1500	1.206±0.018*	29.0
2000	1.212±0.013*	28.7
Catechin 50	1.216±0.029*	25.8
100	1.246±0.006*	26.7

Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates. *p<0.05 when compared with control.

The reducing power (RP) of MEVA is shown in Figure 1. MEVA showed significant (p<0.05) reducing power as indicated by the increase in absorbance value from 0.009±0.001 – 0.242±0.007 at

10 – 1250 µg. Table 1 shows the radical scavenging activity (RSA) of MEVA against DPPH$^{\bullet}$ radical exhibiting maximum RSA of 29.6% on DPPH$^{\bullet}$ radical ($p<0.05$) at 1000 µg. Possible inhibitory effect of MEVA on hydrogen peroxide (HP) is displayed in Table 2. MEVA showed highest percentage inhibition of 85.8% on HP at 20 µg (0.102±0.007) thereafter reducing to 24.2% (0.545± 0.021) at 500 µg. At 50 µg, MEVA (84.8%) compares very well with catechin (83.9%), showing similar scavenging activity. The antioxidant capacity of MEVA against ABTS^{+} radical was investigated in the TEAC (trolox equivalent antioxidant capacity) system as presented in Figure 2.

Table 2: Radical scavenging activity (RSA) of methanolic extract of *Vernonia amygdalina* (MEVA) on Hydrogen peroxides.

Concentration (µg)	Absorbance	% Inhibition
Control	0.719±0.007	-
MEVA 20	0.102±0.007*	85.8
50	0.109±0.008*	84.8
100	0.154±0.003*	78.6
200	0.237±0.006*	67.0
300	0.330±0.006*	54.1
400	0.447±0.004*	37.8
500	0.545±0.021*	24.2
Catechin 50	0.116±0.005*	83.9
100	0.161±0.006*	77.6

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

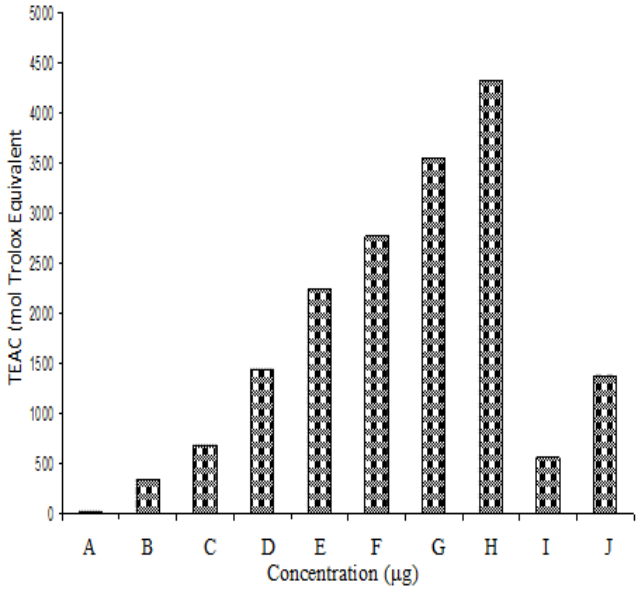

Figure 2: Trolox equivalent antioxidant capacity (TEAC) of methanolic extract of *Vernonia amygdalina* (MEVA). Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates. A=Control, B=20µg MEVA, C=50µg MEVA, D=100µg MEVA, E=200µg MEVA, F=400µg MEVA, G=600µg MEVA, H=1000µg MEVA, I= 50µg catechin, J=100µg catechin.

Table 3: Scavenging effect of methanolic extract of *Vernonia amygdalina* (MEVA) on AAPH-induced lipid peroxidation

Concentration (µg)	Absorbance	% Inhibition
Control	0.087±0.004	-
MEVA 50	0.078± 0.004	10.3
100	0.062±0.003*	28.7
200	0.061±0.0005*	30.0
400	0.073± 0.0001*	16.1
800	0.076±0.001*	12.6
1000	0.081±0.001	6.9
Catechin 50	0.078±0.004	10.3
100	0.058±0.004*	33.3

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Table 4: Potential of methanolic extract of *Vernonia amygdalina* (MEVA) to inhibit deoxyribose degradation by Hydroxyl radical

Concentration (µg)	Absorbance	% Inhibition
Control	0.738±0.006	-
MEVA 50	0.607±0.023*	17.8
100	0.598±0.017*	19.0
200	0.562±0.028*	23.8
400	0.559±0.008*	24.3
600	0.543±0.021*	26.4
800	0.545±0.008*	26.2
1000	0.553±0.008*	25.1
Catechin 50	0.665± 0.015*	9.9
100	0.652±0.002*	11.7

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Table 5: Scavenging effect of methanolic extract of *Vernonia amygdalina* (MEVA) on Nitric Oxide radical

Concentration (µg)	Absorbance	% Inhibition
Control	0.229±0.01	-
MEVA 20	0.179± 0.003*	21.8
50	0.191±0.006*	16.9
100	0.211±0.002*	7.9
200	0.251± 0.002*	-9.6
300	0.287±0.0003*	-25.3
400	0.334±0.007*	-45.9
Catechin 50	0.192±0.001*	16.2
100	0.197±0.001*	14.0

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Likewise, MEVA was able to significantly ($p<0.05$) inhibit AAPH-induced lipid peroxidation by 28.7%, 30.0%, 16.1% and 12.6% at 100, 200, 400 and 800 µg respectively when compared with control (Table 3). Catechin showed greater ability at 100 µg by 33.3% when compared with MEVA (28.7%) at the same concentration. MEVA significantly ($p<0.05$) inhibited OH$^{\bullet}$-induced deoxyribose degradation from 50 – 1000 µg with the highest potency at 600 µg by 26.4% when compared with control as shown in Table 4. Similarly, MEVA exhibited scavenging effect on nitric oxide radical (NO$^{\bullet}$) but at relatively low concentration (Table 5).

Table 6: Correlation Coefficient between Assay methods

Assay methods	**Correlation coefficients (r^2)**
DPPH/RP	0.86
DPPH/LPIA	-0.25
DPPH/TEAC	0.94
DPPH/HP	-0.89
DPPH/HRSA	0.89
DPPH/NO	-0.93
RP/LPIA	-0.57
RP/TEAC	0.97
RP/HP	-0.99
RP/HRSA	0.71
RP/NO	-0.98
LPIA/TEAC	-0.50
LPIA/HP	0.63
LPIA/HRSA	-0.28
LPIA/NO	0.59
TEAC/HP	-0.98
TEAC/HRSA	0.85
TEAC/NO	-0.98
HP/HRSA	-0.76
HP/NO	0.99
HRSA/NO	-0.88

- Negative correlation coefficients

Table 7: Summary of the relationships between Assay methods

DPPH:	RP, TEAC, HRSA
RP:	DPPH, TEAC, HRSA
TEAC:	DPPH, RP, HRSA
HRSA:	DPPH, RP, TEAC
LPIA:	HP, NO
HP:	NO, LPIA
NO:	LPIA, HP

Very strong linear relationships (R^2) were observed between DPPH, RP, TEAC and HRSA while medium to low relationship was observed between LPIA, NO and HP. The level of correlation coefficients between the assays methods are shown in Table 6 while Table 7 summarised the relationship between the various methods.

DISCUSSION

Phytochemicals are diverse and complex in nature. As such, the antioxidant activities of plant extracts cannot be evaluated by only a single method. Different antioxidant components are resident in the antioxidant defence system of the body and the antioxidant capacity of these antioxidant components depends to a great extent on which free radical or oxidants are produced in the system (Choi *et al*., 2002; Akinmoladun *et al*., 2010; Jan *et al*., 2013; Tenore and Ciampaglia, 2013). Therefore, various methods used in evaluating the antioxidant activity of various samples can give varying results depending on the specificity of the free radical used as a reactant (Frankel and Meyer, 2001; Prakash, 2001; Aruoma, 2003; Akinmoladun *et al*., 2010; Patel *et al*., 2011; Ramasarma, 2012; Shabbir *et al*., 2013).

In the present study, MEVA was able to reduce the Fe^{3+}/ferricyanide complex to the ferrous form, Fe^{2+}, which was measured as the formation of Perl's Prussian blue colour at 700nm (Chung *et al*., 2002). MEVA at 250 µg (0.059±0.004) when compared with the reference antioxidant, catechin at 50 µg (0.051±0.003) indicates that the reducing power of MEVA is less than that of catechin, but nevertheless potent enough to function as a good electron and hydrogen-atom donor which can react with free radicals to convert them to more stable products and terminate radical chain reaction. Several studies have also reported the reducing power activity of several plants extracts (Rice-Evans *et al*., 1996; Amarowicz *et al*., 2004; Perez-Perez *et al*., 2006; Manian *et al*., 2008; Sen *et al*., 2013; Rahman *et al*., 2014), and this has been linked to the polyphenolic constituents of these extracts. The assay method used for measuring RP correlates excellently with other methods employed in this study, especially, DPPH, TEAC and HRSA at correlation coefficients (r^2) of 0.86, 0.97 and 0.71 respectively and as such is an excellent method for measuring the reductive ability of antioxidants. Amarowicz *et al*., (2004) in their findings also confirmed that there is a good relationship between RP and DPPH (Sen *et al*., 2013; Das *et al*., 2014).

DPPH radical has been used extensively as a free radical to test the reductive ability of extracts or compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods (Porto *et al*., 2000; Manian *et al*., 2008). Antioxidants react with $DPPH^{\bullet}$ by providing electron or hydrogen atom, thus reducing it to 1,1-diphenyl-2-hydrazine (DPPH-H) or a substituted analogous hydrazine. The deep violet colour of DPPH at maximum absorption of 517 nm is changed to light yellow, colourless or bleached product, resulting in decrease in absorption (Miliauskas *et al*., 2004; Koksal *et al*., 2009). MEVA exhibiting significant scavenging effect on DPPH radical between 40 – 1000 µg (19.3% - 29.6%) compares well with catechin though showing less scavenging activity. Likewise, the assay method of DPPH scavenging activity correlates excellently with RP, TEAC and HRSA with correlation coefficients (r^2) of 0.86, 0.94 and 0.89 respectively. The scavenging effect of plant extracts on DPPH has been shown to be related to the phenolic concentration of the extracts (Manian *et al*., 2008; Akinmoladun *et al*., 2010; Sen *et al*., 2013; Das *et al*., 2014), which is believed to contribute to their electron transfer / hydrogen donating ability. It could therefore be suggested that MEVA contains flavonoids with hydroxyl groups that could stabilize free radicals or scavenge their activities and that the method employed is reliable.

The activity of hydrogen peroxide (HP) as an active oxygen species has been reported to come mainly from its potential to produce the highly reactive hydroxyl radical through the fenton reaction: $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\bullet}$ (Namiki, 1990; Farombi *et al*., 2002; Koksal *et al*., 2009; Jeong *et al*., 2010; Ogasawara *et al*., 2014). Therefore, inhibition of HP formation will prevent further generation of radicals. In the present study, MEVA showed highest percentage inhibition of 85.8% on HP at 20 µg (0.102±0.007) thereafter reducing to 24.2% (0.545± 0.021) at 500 µg. At 50 µg, MEVA (84.8%) compares very well with catechin (83.9%), showing similar scavenging activity. The regression analysis of HP versus other methods in the study revealed a very good relationship between HP scavenging activity and nitric oxide scavenging activity (NO) with a correlation coefficient of 0.99, and a moderate relationship with lipid peroxidation inhibition activity with correlation coefficient of 0.35. Although HP on its own is not very reactive, but it could still be toxic both *in vitro* and *in vivo*; it could induce cell death *in vitro* and attack many cellular energy-producing systems *in vivo*. For instance, it has been reported that HP could deactivate the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (Hyslop *et al*., 1998; Koksal *et al*., 2009; Song *et al*., 2014).

$ABTS^+$ radical was generated by oxidation of ABTS with potassium persulfate (Re *et al*., 1999). MEVA increased TEAC values in a dose dependant manner from 50–600 µg with 339.4±155.3 – 4320.0±33.6 trolox equivalent and shows almost equal potency with catechin at 50 µg. $ABTS^+$, a more reactive radical (more reactive than $DPPH^{\bullet}$) is stabilized by antioxidants that can supply electrons, unlike H atom in $DPPH^{\bullet}$. MEVA was thus able to scavenge $ABTS^+$ radical-induced peroxodisulfate formation by electron transfer. The regression analysis for the assay methods employed in this study reveals that measurement of $ABTS^+$ scavenging activity as Trolox equivalent antioxidant capacity (TEAC) compares very well with other methods in showing strong linear correlation with DPPH, RP and HRSA with correlation coefficients (r^2) of 0.94, 0.97 and 0.85 respectively. This indicates that TEAC is a reliable method for measuring the total antioxidant activities of substances as reported by previous works (Koksal *et al*., 2009; Piljac-Zegarac *et al*., 2009; Chohan *et al*., 2012). Likewise, the report of Sasikumar *et al*., (2010) supported the relationship between TEAC and DPPH.

Lipid peroxidation is one of the consequent actions of ROS and free radicals. In this study, lipid peroxidation was induced with AAPH, a peroxy radical initiator. Thermal decomposition of AAPH produces peroxy radical which can attack polyunsaturated lipids initiating lipid peroxidation (Neergheen *et al*., 2006). MEVA was able to significantly ($p<0.05$) inhibit AAPH-induced lipid peroxidation by 28.7%, 30.0%, 16.1% and 12.6% at 100, 200, 400 and 800 µg respectively when compared with control. Catechin showed greater ability at 100 µg by 33.3% when compared with MEVA (28.7%) at the same concentration.

The decreasing lipid peroxidation product measured as thiobarbituric reactive substances (TBARS) showed the ability of MEVA to inhibit AAPH-induced lipid peroxidation by 10.3% – 30.0%. LPIA (Lipid peroxidation inhibitory activity of MEVA) correlates fairly well with HP and NO; the other methods employed in this study, with correlation coefficients (r^2) of 0.63 and 0.59 respectively and as such, LPIA is another reliable method of measuring the scavenging and inhibitory ability of antioxidants. In the report of Akinmoladun *et al*., (2010), very low to fair correlations were observed between LPIA and other methods, which is similar to what was observed in this study.

Furthermore, the antioxidant activity of MEVA was demonstrated against hydroxyl radical ($OH^{\bullet}$). $OH^{\bullet}$ has been reported to be the most reactive radical known; it attacks and damages almost every molecule it makes contact with (Halliwell, 1989; Aruoma, 1999; Manian *et al*., 2008; Ramasarma, 2012). $OH^{\bullet}$ rapidly reacts with any molecule, forming another radical species, thereby giving rise to chain peroxide formation. The deoxyribose method employed in this study is a simple assay to determine the rate constants of hydroxyl radical formation as reported by Halliwell *et al*., (1987). $OH^{\bullet}$ radical is generated when the mixture of $FeCl_3$-EDTA, H_2O_2 and ascorbate is incubated with deoxyribose in phosphate buffer (pH 7.4). $OH^{\bullet}$ attack on deoxyribose results ultimately in chain peroxidation which could be measured as TBARS. MEVA exhibited 17.8 – 26.4% inhibition of deoxyribose oxidation by $OH^{\bullet}$. The ability of MEVA to inhibit $OH^{\bullet}$ by preventing the oxidation of deoxyribose in the current *in vitro* model could be related to prevention of propagation of the process of lipid peroxidation *in vivo* as has been shown in our studies with CCl_4 (Adesanoye and Farombi, 2010). In this vein, Farombi *et al*., (2002) reported that the overall antioxidant effect of flavonoids on lipid peroxidation is the result of scavenging hydroxyl radicals and superoxide anions at the stage of initiation and termination of peroxyl radicals according to Hussain *et al*., (1987). In addition, MEVA showed better inhibition potential of 17.8% against 9.9% for catechin at 50 µg. The regression analysis in this study revealed a very high relationship between hydroxyl radical scavenging activity (HRSA) and other methods which is in line with some previous works (Perez-Perez *et al*., 2006; Sazikumar *et al*., 2010). HRSA correlated excellently

with DPPH, RP and TEAC, with correlation coefficients (r^2) of 0.89, 0.71 and 0.85 respectively. The relationship between these four methods is excellent as has been observed and reported by various scientists (Halliwell, 1989; Amarowicz *et al*., 2004; Manian *et al*., 2008; Piljac-Zegarac *et al*., 2009; Akinmoladun *et al*., 2010).

Similarly, MEVA exhibited scavenging effect on nitric oxide radical ($NO^{\bullet}$) but at relatively low concentration. $NO^{\bullet}$ shows dual function as both an antioxidant and pro-oxidant depending on the relative ratios of the reactants (Aruoma, 1996; Groß *et al*., 2013; Meenakshi and Agarwal, 2013). Antioxidant effects of $NO^{\bullet}$ occurs when $NO^{\bullet}$ reacts with alkoxy and peroxyl radical intermediates during lipid peroxidation thereby stabilizing the inhibition of LDL oxidation while the pro-oxidant reaction occurs when $NO^{\bullet}$ reacts with $O_2^{\bullet}$ to yield peroxynitrite ($ONOO^{\bullet}$) (Zielonka et al., 2010). This dual effect could explain why the scavenging effect on $NO^{\bullet}$ decreased with increasing concentration of the extract and became pro-oxidant at concentrations greater than 100 µg. MEVA exhibited similar scavenging effect with catechin. At 50 µg, MEVA elicited 16.9% inhibition while catechin showed 16.2% inhibition potential. The ability of MEVA to scavenge $NO^{\bullet}$ and especially its deleterious metabolite, ($ONOO^{\bullet}$) will be highly beneficial in biological system as $ONOO^{\bullet}$ and some other $NO^{\bullet}$ metabolites have been implicated in various pathological conditions such as malaria, cardiovascular diseases, inflammation, cancer and diabetes (Aruoma, 1996; Groß et al., 2013; Meenakshi and Agarwal, 2013). NO correlated with LPIA and HP with correlation coefficients (r^2) of 0.59 and 0.99 respectively.

CONCLUSION

In conclusion, this study demonstrated that MEVA is a potent antioxidant and scavenger of reactive species *in vitro*. The observation concerning the diversity and complexity of the phytochemicals in plant extracts with different mechanisms of reaction for specific antioxidant species applies very well to the present study. The antioxidant capacity observed by all the methods assayed in this work appears to be reliable for measuring antioxidant and scavenging capacity of substances. From various experimental results (Dasgupta and De, 2004; Perez-Perez *et al*., 2006; Akinmoladun *et al*., 2010; Rao *et al*., 2010; Sasikumar *et al*., 2010; Patel *et al*., 2011; Ramasarma, 2012; Shabbir *et al*., 2013), a linear correlation with high correlation coefficients has been observed between total phenolic/flavonoid content and various antioxidant and free radical scavenging activities of various plants indicating that the various activities are based on the flavonoid and phenolic contents of the plant materials. It could therefore be inferred that the activities of MEVA in this study is due to its phenolic content, especially the flavonoids. Therefore, MEVA could be recommended as a potential antioxidant in ameliorating oxidative and free radical-induced pathologies.

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Comparison of Outcome of Students' Performance Using the Standard Setting Method with the Absolute Grading Method in Preclinical Examination

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Summary: This study compared the outcome of students' performance using the standard setting method with the equivalent outcome they would have obtained using the absolute grading method. It involved the comparison of fail, pass, honors and distinction grades in Digestive System, Endocrine System, Cardiovascular System and Health and the Environment courses in the MBBS Stage I examination. The performance in Cardiovascular System was significantly better with the standard setting method ($\chi^2 = 27.53$; $p < 0.01$), median score in the honors range compared with the absolute grading method where the median score was in the pass range. On the other hand, the performance in Endocrine System was significantly better using the absolute grading method ($\chi^2 = 27.30$; $p < 0.01$), with median score in the honors range compared with the standard setting method where the median score was in the pass range. There was no difference in the performance in Digestive System ($\chi^2 = 7.45$; $p = 0.06$), median score in the pass range and Health and the Environment ($\chi^2 = 6.34$; $p = 0.09$), median score in the honors range; between the standard setting and absolute grading methods (Wilcoxon's signed rank). The overall pass, honors, distinction and failure rates were also identical in both methods (Mann Whitney U test). This suggests that overall the outcome of the students' performance in the standard setting method compared with the absolute grading method were not significantly different.

Keywords*:* standard setting, absolute grading method, students' performance, preclinical education.

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INTRODUCTION

The Faculty of Medical Sciences at the University of the West Indies (UWI) awards the Bachelor of Medicine and Bachelor of Surgery (MBBS) degree after five years of study. The MBBS Stage I examination is the final examination taken at the preclinical level before the students proceed to the last two years of their clinical training. It is equivalent in content and scope to the United States Medical Licensing Exam (USMLE) Step 1 examination.

At the Mona Campus, there has been significant curriculum reform, which includes a change from discipline-based to systems-based teaching in the preclinical years, with early clinical-preclinical integration. A part of this reform was a change in the marking system from the absolute grading to the standard setting method (Branday and Carpenter, 2008).

The aim of standard setting is to minimize errors in determining the pass/fail, honors and distinction cut off points while accounting for the varying difficulty of the examinations. Valid and reliable tools for assessing the quality of medical education are central to high quality medical care (Boursicot and Roberts, 2006). A standard may be absolute or criterion- referenced, where it is based on pre-determined criteria, irrespective of examinee performance or relative, that is, norm-referenced, where it is dependent on the performance of the particular group of examinees (Bandaranayake, 2008).

Standard setting is the process of deciding "what is good enough" and the process used to arrive at such decision is paramount (Cusimano, 1996). A standard is a special score that serves as a boundary between those who perform well enough and those who do not (Norcini, 2003). The standard setting grades define the boundary between passing and failing, that is, it separates candidates who are competent from those who are not. Additionally, the cutoff marks to achieve honors and distinction grades can also be decided by this process. The Angoff method of standard setting is currently being used at the Faculty of Medical Sciences, The University of the West Indies, Mona Campus. It is objective, has the advantages of being used in a range of licensing

and certifying examinations and well supported by research. However, it can be very labor intensive and time consuming (George et al, 2006).

The absolute grading system, on the other hand, is based on the idea that grades should reflect mastery of specific knowledge and skills. The teacher sets the criteria for each grade and all students who perform at a given level receive the same grade. This system is a "percent of total points possible" and it is assumed that a student who scores 85% knows 85% of the material. Traditionally, in the old curriculum at UWI using the absolute grading method, students achieving a score of 50% and above passed the examination while those scoring 65% and over were awarded honors and those with scores above 75% were awarded distinctions. One weak point associated with the absolute grading system is the rationale for the cut-off or pass/fail scores that is not based on any analysis (Grading Systems, 1991).

We hypothesized that there was no difference in the outcome of students' performance that is pass/fail, honors and distinction cut off marks, using the standard setting method when compared with the absolute grading method. Based on the foregoing therefore, the present study was designed to compare the outcome performance on the MBBS Stage I examination of preclinical students at the Mona Campus using modified Angoff method of standard setting compared with the equivalent outcome they would have obtained using the absolute grading method.

MATERIALS AND METHODS

In this retrospective study, we collected from the office of the MBBS Program Director the standard setting grades of all preclinical students who took the MBBS Stage I examination in December 2007. There were 131 students who sat for the Endocrine System and Health and Environment courses of the exam, and 130 students who sat for the Digestive System and Cardiovascular System courses. The examination was made up of 50 multiple choice questions (MCQ) in the Digestive System course with a duration of one hour; 75 MCQ in Health and Environment course with a duration of 1.5 hours and 100 MCQ in both Endocrine System and Cardiovascular System courses with a duration of 2 hours each. All scores were converted to a 100% scale score. They were compared with equivalent scores that the students would have obtained via the absolute grading method.

At the Mona campus, the modified Angoff method of standard setting is used.

In this method, a minimum of eight judges made up of experienced lecturers in the different departments who were involved in teaching a particular course formed the panel for that course. The different systems are "team taught" by lecturers from the respective disciplines. The judges had undergone a two day training session in standard setting method. They examine each multiple choice question with a four option choice to select the correct answer from and estimate the probability that the "minimally competent" or "borderline" candidate would answer the item correctly. The lecturers answer the questions within the time allotted for the examination and each judge's estimate scores on all items are added up and averaged and the test standard for that course is the average of these means for all the judges. Questions that were either ambiguous or poorly framed were corrected, while those that were either too difficult or too easy were replaced. The judges therefore determine the pass mark and also the marks required to achieve honors and distinction.

We analyzed the data using the SPSS statistical package for medians, range, Wilcoxon's signed rank test for two (paired) related data, the Mann Whitney U test and Chi squared test with $p < 0.05$ taken as the level of statistical significance. The study was approved by the Ethics Committee of the Faculty of Medical Sciences, The University of the West Indies, Mona Campus.

RESULTS

In the standard setting system, the median score of 60% for Digestive System and 66% for Endocrine System were in the pass range while it was in the honors range for Cardiovascular System (63%) and Health and Environment (67%). With the absolute grading method, the median score of 61% for Digestive System and 62% for Cardiovascular System were in the pass range while it was in the honors range for Endocrine System (65%) and Health and Environment (69%). The performance in Cardiovascular System was significantly better with the standard setting method ($\chi^2 = 27.53$; $p = 0.01$), median score being in the honors range; compared with the absolute grade method where the median score was in the pass range. On the other hand, the performance in Endocrine System was significantly better using the absolute grade method ($\chi^2 = 27.30$; $p = 0.01$), with median score in the honors range, compared with the standard setting method where the median score was in the pass range. There was no difference in the performance in Digestive System ($\chi^2 = 7.45$; $p = 0.06$) with the median in the pass range, and Health and Environment ($\chi^2 = 6.34$; $p = 0.09$), with the median in the honors range, between the standard setting and absolute grading methods (Wilcoxon's signed rank). The overall pass, honors, distinction and failure rates are also similar in both methods (Mann Whitney U test) (Table 1).

Table 1. Outcome of students' performance using the standard setting method and the absolute grading method

Courses	Standard setting	Absolute grading	Chi – square
Digestive System (N = 130)	F (0-46%) = 8 P (47-64%) = 77 H (65-78%) = 42 D (79-100%) = 3 Median = 60% Range: 34-84%	F (0-49%) = 16 P (50-64%) = 67 H (65-74%) = 37 D (75-100%) = 10 Median = 61%	$\chi^2 = 7.48$ p = 0.06
Endocrine System (N = 131)	F (0-45%) = 2 P (46-49%) = 92 H (70-78%) = 33 D (79-100%) =4 Median = 66% Range: 42-87%	F (0-49%) = 7 P (50-64%) = 53 H (65-74%) = 52* D (75-100%) = 19 Median = 65%	$\chi^2 = 27.30$ p = 0.01
Cardiovascular System (N = 130)	F (0-42%) = 3 P (43-56%) = 40 H (57-66%) = 46* Median = 63% Range: 49-79%	F (0-49%) = 1 P (50-64) = 50 H (65-74) = 55 Median = 62%	$\chi^2 = 27.53$ p = 0.01
Health & Environment (N = 131)	F (0-49%) = 1 P (50-67%) = 70 H (68-77) = 43 D (78-100%) = 17 Median: 67% Range: 49-79%	F (0-49%) = 1 P (50-64) = 50 H (65-74) = 55 D (75-100) = 25 Median: 69%	$\chi^2 = 6.33$ p = 0.09
Summary			
Failure rate	4%	10%	
Pass rate	47%	43%	
Honors rate	35%	32%	
Distinction rate	14%	15%	

F = Fail; P = Pass; H = Honors; D = Distinction *p < 0.01

DISCUSSION

The result of the present study showed that overall there were no significant differences in the outcome of the student' performance in all the four courses examined between the standard setting and absolute grading methods. This might also suggest that the two methods of assessment test identical knowledge base of the students. The outcome of the students' performance in Cardiovascular System was better with the standard setting method while in Endocrine System it was better with the absolute grading method. Their performance in Digestive System and Health and Environment were identical in both methods.

It is interesting to observe that the pass score mark set with the standard setting method in three of the four courses is less than the fifty percent pass mark used in the absolute grading system. However, the score required to achieve a distinction grade with the standard setting method is higher in the standard setting method than in the absolute grading method in three of the four courses examined. This might suggest that the standard setting method favors more students passing an examination but require a bit more from them to achieve distinction grade.

The overall pass rate (i.e. students who obtained pass, honors and distinction grades) of 96% in the standard setting method in the present study is similar to the 100% pass rate reported by George et al (2006) using the modified Angoff method. Also there is no difference between the 96% pass rate for the standard setting method and the 90% pass rate for the absolute grade method in the present study.

Impara and Plake (1998) had suggested that most of the judges often find it difficult to accurately conceptualize borderline candidates. The variability seen in the cut off pass/fail, honors and distinction marks of the respective courses could be attributed to the varied perceived level of difficulty by the different judges.

The process of standard setting, although very labor intensive and time consuming is justified because of its usefulness to the students, faculty and university at large as part of an internal quality assurance process intended to improve the validity of pass/fail, honors and distinction scores, bring teachers together to discuss course objectives, relevance and assessment. This is in contrast to the absolute grading method where the pass/fail, honors and distinction scores are pre-determined based on traditional or historic antecedents.

The examination format being used presently at the MBBS Stage I of the Faculty of Medical Sciences University of the West Indies, Mona Campus is the multiple choice. One set back of this examination format is that graduates produced mainly through the

multiple-choice standard setting format, without written or essay examinations may be deficient in communication, especially in writing (Bassaw and Pitt-Miller, 2007).

In conclusion, there is no significant difference in the outcome of the performance of the students between the absolute grading and standard setting methods suggesting that they might be testing identical knowledge base. The use of the standard setting method is still in its infancy in many medical schools and there are others yet to adopt this method of assessment. The adoption and continued usage of the standard setting method is supported because of its objectivity and validity in setting the pass/fail, honors and distinction scores and its usefulness in quality assurance and bringing teachers together to discuss course objectives and assessment.

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Short Communication

Effect of Chronic Administration of Methanol Extract of *Moringa Oleifera* on Some Biochemical Indices in Female Wistar Rats

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Summary: The study was conducted to investigate safety associated with prolonged consumption of *Moringa oleifera* leaves as beverage. Fourteen rats were used in this study. They were divided into 2 groups each containing 7 rats. Rats in group I received 2ml/kg of corn oil (standard vehicle drug). Animals in groups II were administered with 400mg/kg body of methanolic extract of *Moringa oleifera* (MEMO) for five weeks respectively. Serum collected was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, blood urea nitrogen (BUN) and creatinine. There was significant ($P<0.05$) decrease in serum total protein, albumin, globulin and AST activity. The activity of ALT decreased but not significant. Similarly, 400mg/kg body of MEMO led to significant ($P<0.05$) decrease in serum BUN and creatinine. All experimental animals that received 400mg/kb of MEMO had significant ($P<0.05$) decrease in body weight from week to week 4 of the experiment. Taken together, 400mg/kg body of MEMO seemed to be toxic to the liver with apparently no toxicity in the kidney. Hence, prolonged exposure is not advisable as such could portend danger to the liver.

Keywords*: Moringa oleifera*, toxicity, hepatic damage, kidney.

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INTRODUCTION

Moringa oleifera Lam. (drumstick tree, horse-radish tree, synonym: *Moringa pterygosperma* Gaertn.), a member of the family Moringaceae, is a small-medium sized tree, 10–15m high, widely cultivated in East and Southeast Asia, and the West Indies. *Moringa oleifera* is known to tolerate a wide range of rainfall with minimum annual rainfall requirements which has been estimated at 250 mm and maximum at over 3000 mm and a pH of 5.0–9.0 (Palada and Changl, 2003). Different parts of the *Moringa oleifera* tree are reported to possess various pharmacological actions and nutritional qualities (Viera *et al.*, 2010; Promkum *et al*., 2010; Oluduro *et al.*, 2011; Debnath *et al.*, 2011 ; Ogunsina *et al*., 2011;). The leaves and fruits are found to have hypocholesterolaemic activity in Wistar rats and rabbits, respectively (Ghasi *et al*., 2000; Mehta *et al*., 2003). Similarly, the flowers and roots are used in folk remedies, for tumours, the seeds for abdominal tumours, leaves applied as poultice to sores, rubbed on temples for headaches and are said to have purgative properties (Anwar *et al*., 2007). The leaves as well as flowers, roots, gums and fruits are extensively used for treating inflammation (Cheenpracha *et al.*, 2010) and cardiovascular diseases (Chumark *et al*., 2008). Also, its leaves can serve as a rich source of beta-carotene (Nambiar and Seshadri, 2001), vitamin C and E, and polyphenolics (Sreelatha and Padma, 2009). *Moringa oleifera* has been reported for its potent antioxidant and free radical scavenging activities in vitro and in vivo (Singh *et al*., 2009; Sreelatha and, Padma, 2009; Rermal *et al.*, 2009; Atawodi *et al.*, 2010).

The use of computer modelling software package to evaluate safety and risk assessment of phytochemicals in experimental animals has been recently reported (Valerio *et al*., 2010). However, information is lacking on safety and toxicological evaluation of *Moringa oleifera* in animal models. Therefore, this study was designed to elucidate the possible toxicity associated with chronic administration of methanolic extract of Moringa oleifera (MEMO) in female rat model.

MATERIALS AND METHODS

Sodium hydroxide (NaOH), copper sulphate, potassium chloride, sodium- potassium tartarate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and

were obtained from the British Drug Houses (Poole, Dorset, UK) and Randox.

Preparation of plant extract

Fresh leaves of *Moringa oleifera* (MEMO) were harvested from Ajibode extension of the University of Ibadan, Oyo State, Nigeria. The coarse powder (1.2kg) of the leaves was defatted using N-hexane (60-80^{O}C) before being extracted with 95% methanol in a soxhlet extractor at 55 ^{O}C for 6 h. The extract was concentrated under reduced pressure to yield a syrupy mass (40g) and stored in a cool place until the time of use.

Experimental design and animal treatment

Thirty healthy adult female Wistar rats weighing between 210-230g obtained from the Central Animal House, University of Ibadan, Nigeria were randomly assigned to five groups of 6 animals per group. They were housed in plastic suspended cages placed in a well-ventilated rat house, provided rat pellets and water *ad libitum* and subjected to natural photoperiod of 12-hr light : dark cycle. Rats in group I served as control and were administered 2ml/kg body weight of corn oil according to Farombi *et al.* (2007). Animals in group II received 400mg/kg body of methanolic extract of *Moringa oleifera* (MEMO) for five weeks consecutively. The animals were sacrificed at the end of the eight weeks administration by cervical dislocation after an overnight fast. About 5ml of blood was drawn into plain sample bottles and allowed to clot. Clotted blood was later centrifuged at 3,000 revolutions per minutes (RPM) and the clear serum was harvested.

Animal ethics

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (PHS, 1986). The experiment was carried out at Biochemistry Laboratory, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Serum Biochemistry

Protein concentration was determined by the method of Lowry *et al*. 1951. Serum sample (1ml) was dissolved in 39ml of 0.9% saline to give a 1 in 40 dilution. This was followed by the addition of 3ml of Biuret reagent to the sample.

The mixture was incubated at room temperature for 30 minutes after which the absorbance was read at 540nm. And the protein content of sample was thereafter calculated from the standard using Bovine Serum Albumin (BSA). AST (EC 2.6.1.1) activity was assessed according to Reitma and Frankel (1957). Briefly, 0.1ml of diluted serum was mixed with phosphate buffer (100mmol/L, pH 7.4), L-aspartate (100mmol/L), α-oxoglutarate (2mmol/L) and the mixture was incubated for exactly 30min at 37^{o}C. 0.5ml of 2, 4-dinitrphenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20min at 25^{0}C. Then 5.0ml of NaOH (0.4mol/L) was added and the absorbance read against the reagent blank after 5 min at 546nm. AST was measured by monitoring the concentration of oxaloacetate-hydrazone formed with 2, 4-dinitrophenylhydrazine. ALT (EC 2.6.1.2) activity was determined following the principle described by Reitman and Frankel (1957), 0.1ml of diluted serum was mixed with phosphate buffer (100mmol/L, pH 7.4), L-alanine (100mmol/L), and α- oxoglutarate (2mmol/L) and the mixture was incubated for exactly 30 mins at 37^{o}C. 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) was then added to the reaction mixture and allowed to stand for exactly 20 mins at 25^{o}C. Then 0.5ml of NaOH (0.4mol/L) was added and the absorbance was read against reagent blank after 5 mins. Reagent blank was prepared as described above replacing sample with 0.1ml of distilled water. ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nm. Albumin was measured with Bromocresol green, a dye at an acid pH of 3.8 preferentially bound albumin to produce a shade of green colour which was measured with a spectrophotometer at 630nm (Gustafsson, 1976). Serum blood urea nitrogen (BUN) and creatinine levels were assesses using Randox kits according to manufacturer's instruction. The blood urea nitrogen (BUN) and creatinine levels in all the sample sera were estimated by modified methods based on diacetylmonoxime reaction (Marsh *et al.*, 1965) and Jaffe's reaction (Biod and Sirota, 1948), respectively, on standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.).

Statistical analysis

All values are expressed as mean±S.D. The test of significance between two groups was estimated by Student's t test. "One-way ANOVA with Dunnett's post-test was also performed using GraphPad Prism version 4.00.

RESULTS

There was significant ($P<0.05$) decrease in weight gain in rats that received 400mg/kg of MEMO weeks 1 and 2 of administration of MEMO compared with the control values (fig 1). At 5 weeks of administration, rats that received the extract had significant increase in weight gain (230.00±10.00) compared with the control (218.00±12.41) respectively. Serum total protein (3.62±0.20), albumin (1.10±0.03) and globulin (2.50±0.22) obtained in animals that were administered with the

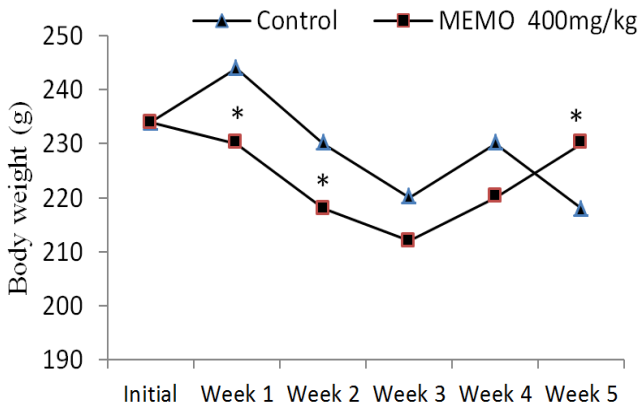

Figure 1: Effect of prolonged administration of MEMO on weekly weight gain. n=8, *p<0.05.

extract reduced significantly at p<0.05, p<0.001 and p<0.001 respectively compared with the control total protein (5.58±0.22), albumin (2.18±0.08) and globulin (3.88±0.22) respectively (Table 1). The increase in ALT obtained in rats administered with 400mg/kg of MEMO was not significantly (p>0.05) different from the control. Similarly, serum AST activity in rats administered with the extract fell (p<0.001) significantly when compared with the control. Serum creatinine and blood urea nitrogen (BUN) which are markers of kidney damage reduced (p<0.05) significantly in animals exposed to MEMO compared with control (table 1) respectively.

DISCUSSION

In our study, total protein, albumin and globulin decreased significantly in animals exposed to 400mg/kg of MEMO for five weeks. The decrease observed in these serum proteins was a good pointer to hepatic damage due to reduced capability of the hepatocytes to synthesize enough serum proteins. Normal albumin in the bloodstream is important for maintaining many physiologic functions in the body. One of these functions is critical for maintenance of fluid pressure in the arteries and veins. When the protein level falls below a certain point, the fluid in these vessels can leak out and pool in the abdominal or thoracic cavities. Assay of serum albumin level is often considered as a test for liver function. The hepatic synthesis of albumin has been reported to decrease significantly in end-stage liver disease (Sherwin *et al*., 1996). In this study, 400mg/kg of MEMEO caused significant decrease in serum albumin level. Our findings are in agreement with the reports of other authors that reduced serum/plasma albumin was associated with hepatic damage (Shin *et al*., 2010; Yousef *et al*., 2010). According to our study, we also observed a significant decrease in serum total protein in rats that were administered with 400mg/kg of MEMO. Decrease in serum total protein is known to be associated with defective capability of the hepatocytes to synthesize proteins. Protein synthesizing potentials of the hepatocytes have been extensively documented (Sathesh *et al*., 2009; Ahmed and Urooj, 2010; Najmi *et al*., 2010) to crash significantly following hepatic injury/damage.

Cellular leakage of enzymes occurs often when the cell architecture and integrity is damage. Accordingly, the presence of enzymes in the serum above their normal level is a pointer to clinical diagnosis of variety of pathological conditions. Serum aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) have been reported by numerous authors as markers for acute and chronic hepatocellular damage (Dufour *et al*., 2000a; Dufour *et al*., 2000b). Pyridoxal-5-phosphate (PLP) which is the active form of vitamin B6 is the coenzyme for both ALT and AST respectively (Rej, 1977). Significant decrease in serum AST might be due to metabolic, drug-induced or iatrogenic (unknown cause). Pathophysiological conditions associated with deficiency of vitamin B6 might lead to decrease in serum AST and ALT activities or underestimation of their activities if optimized methods are not used (Lum, 1995). Decrease in the serum/plasma activities of aspartate aminotransferase has been shown to correlate with pyridoxal-5-phosphate deficiency (Waner and Nyska, 1991; Evans and Whitehorn, 1995; Hall, 2001; Saori *et al*., 2003). Different authors have found significantly lower in vivo serum concentrations of PLP in epileptic patients treated with anticonvulsant drugs (Young, 2000; Apeland *et al*., 2002; Apeland *et al*., 2003). The animals administered with MEMO had significant reduction of body weight. This effect might be potentially beneficial for the obese individual addicted to high calorie intake. The mechanism(s) of loss of body weight could be through loss of body fat (Palit *et al*., 1999) or decrease in serum cortisol level (Garrison and Chambliss, 2006).

Blood urea nitrogen (BUN) produced in the liver is derived from the diet or tissue sources and is excreted in the urine via the kidney. Serum urea accumulates in the serum in renal disease when the

Table 1: Effect of prolonged administration of MEMO on serum liver and kidney function tests

Treatment	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	ALT (U/L)	AST (U/L)	BUN (mg/DL)	Creatinine (mg/DL)
Control	5.58±0.22	2.18±0.08	3.88±0.22	58.60±0.75	17.33±1.63	1.25±0.01	1.23±0.02
MEMO	3.62±0.20*	1.00±0.03$^{\#}$	2.50±0.22$^{\#}$	58.67±1.33	10.75±0.95$^{\#}$	1.08±0.03*	1.07±0.06*

Values expressed as mean±S.D, n=8, *p<0.05, $^{\#}$p<0.001

rate of production exceeds that of excretion (Mayne, 1994). Serum creatinine is basically derived from endogenous sources by tissue creatinine breakdown (Mayne, 1994). Therefore, Elevation of urea and creatinine levels in the serum had been taken as the index of nephrotoxicity (Ali *et al.,* 2001; Flaoyen *et al*., 2001). In our study, there was a clear significant reduction in both serum creatinine and urea in rats exposed to prolonged administration of MEMO. This shows that MEMO is not toxic to the kidney. Interestingly, the results obtained on the liver function tests were opposite to that of the metabolites associated with kidney function test. According to this study, prolonged use of MEMO was toxic to the liver and not to the kidney. Our previous report showed that MEMO at 400mg/kg was toxic both to the liver and the kidney in male Wistar rats (Oyagbemi et al., 2013). Surprisingly, this was the opposite in female Wistar rats. However, the use of *Moringa oleifera* leaves as beverages in sub-Saharan Africa should be minimized as prolonged exposure to this medicinal plant could probably precipitate liver damage.

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Erythrocyte Osmotic Fragility and Excitability Score in Rabbit fed *Hibiscus Sabdariffa* in Graded Level

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Summary: This study was conducted for 10 weeks with the aim of investigating the erythrocyte membrane integrity as measured by erythrocyte osmotic fragility and excitability scores of rabbits fed graded level of *Hibiscus sabdariffa* calyx (HSC). Twenty weaners' rabbit of both sexes were used for the study and were placed on four experimental diets which contain the following percentages of HSC 0 %, 25 %, 50 %, 75 %, as feed additive and were added at 0 g, 62.5 g, 125 g, 187.5 g designated as T_1, T_2, T_3 and T_4 experimental diets. Excitability scores were measured weekly as described by Voisnet *et al*. (1997). At the end of the experiment, the rabbits were slaughtered by severing the jungular vein. A Blood sample (2 ml) was collected from each rabbit into sampled bottles, containing the Na EDTA as anticoagulant for hematological analysis. Packed cell volume (PCV) Haemoglobin concentration (Hb), Total red blood cell (RBC) count, Total leukocyte count as well as differential leukocyte was determined using standard method. The percentage haemolysis recorded at 0.3 % to 0.8 % was significantly ($P < 0.05$) higher in rabbits in T_1 compared to the remaining 3 diets. The result of excitability score shows that rabbit on diet 1 and 2 had a lower value which was significantly ($P < 0.05$) lower than rabbits on diets 3 and 4 with a value of 65.5 ± 5.0 and 70.00 ± 5.50 % respectively. In conclusion this study demonstrated for the first time that chronic administration of HSC improves haematological parameters, brain mood and function as well as maintaining erythrocyte membrane integrity.

Keywords: Erythrocyte osmotic fragility, Excitability score, *Hibiscus sabdariffa*, Rabbits, Haematological parameters.

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INTRODUCTION

Hibiscus sabdarifa calyces (HSC) is a common beverage drink in Nigeria, and it possesses some medicinal properties, the extract has been reported to contain 17 amino acid and possess antibacterial (Oboh, 2004) and antipyretic activities (Ali *et al*., 20005). It has also been shown to protect cells against oxidative stress in rats (Wang *et al.*, 2000) and increases immune-modulating factors (Muller and Franz, 1992; Ismail *et al*., 2008). The medicinal and nutritive values of calyces of HSC suggest that it could be used in ameliorating the adverse effect on heat stress in poultry (Minka *et al*. (2007) because it was reported by Wang *et al.* (2000) and Essa *et al.* (2006) that it contain flavonoid anthocyanin and vitamin C and there are indications as postulated by several authors that extracts from the calyces of HSC contain antioxidant principles (Wang *et al*., 2000; Tee *et al*., 2002; Ologundudu and Obi, 2005; Ologundudu *et al*., 2009). The extract has been demonstrated to protect cells against oxidative stress in rats (Wang *et al*., 2000) and against oxidative tissue damage (Wolff *et al.*, 1986). Haematological and behavioural parameters have been used to assess the degree of stress and also the health status in livestock (Adenkola and Ayo, 2010).

This study was therefore design to investigate the excitability and erythrocyte osmotic fragility of rabbits fed graded level of HSC.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at Federal Housing Estate in North Bank Makurdi (07^0 $41^/$ N, 08^0 $37^/$ N) in the Southern Guinea Savannah Zone of Nigeria.

Experimental Animals and Management

The study was conducted using a total number of twenty weaners' rabbit of both sexes which were obtained within Makurdi metropolis in a study which lasted 10 weeks. The animals were randomly allocated to four dietary treatments groups with five rabbits per treatment in a completely randomized design. The cages were kept in an open sided building for easy and effective cross-ventilation, and the cages were washed thoroughly and disinfected at least two weeks before the animals were brought in. The rabbits were kept for seven days prior to the commencement of the study, during which the animals were accustomed to routine handling, and

acclimatize to the new environment, as well as to stabilize them from the stressful effect of road transportation (Adenkola *et al*, 2011) which they may have been subjected to from where they were purchased to the experimental site.

Experimental Design

The animals were allocated to four dietary treatments. The four experimental diets were formulated contain the following percentages of HSC 0 %, 25 %, 50 %, 75 %, was added at 0 g, 62.5 g, 125 g, 187.5 g for T_1, T_2, T_3 and T_4 experimental feed (Table 1).

Determination of Haematological Parameters

At the end of the experiment, the rabbits were slaughtered by severing the jungular vein. Blood samples (2ml) was collected as the rabbits bled into sampled bottles, containing the anticoagulant, sodium salt of ethylene, diaminetetraacetic acid at the rate of 2 mg/ml of blood (Adenkola and Ayo, 2009). After collection, the blood samples were taken to the physiology laboratory, in the department of veterinary physiology and pharmacology, University of Agriculture Makurdi, for hematological analysis, in which packed cell volume (PCV) was determined using microhaematocrit method, haemoglobin concentration (Hb) using the cyanomethaemoglobin method, total red blood cell (RBC) count, total leukocyte (WBC) count and differential leukocyte was also determined using the haemocytometric method (Schalm *et al* 1975).

Erythrocyte Osmotic Fragility Determination

Sodium chloride solution was prepared according to Faulkner and King (1970) in volume of 200ml for each of the samples in concentration ranging from 0.1 to 0.85 at pH 7.4. a set of 10 test tubes, each containing 5 ml of sodium chloride solution of concentration ranging from 0.1 to 0.85 %, where arranged serially in a test tube rack. One set of the test tube was used to analyze each sample. The test tubes were labeled with corresponding sodium chloride concentrations. A drop of blood was dropped into each of the ten test tubes using a syringe. The content was then mixed by gently inverting the test tubes for about 3 times. The test tubes were allowed to stand at room temperature (26 - 27^0 C) for 20 minutes. The contents of the test tubes were maintained at pH 7.5 thereafter the contents of the test tube were centrifuged at 1,500 g for 20 minutes. The supernatant of each test tube was transferred into a cuvette. The concentration of haemoglobin in the supernatant solution was measured at 540 nm using a spectrophotometer by reading the absorbance. The same procedure was repeated for every blood sample used for the study. The percent haemolysis was then calculated using the formula (Faulkner and King, 1970):

$$\text{Percent Haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} X\ 100$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the sodium chloride concentrations.

Measurement of Excitability Scores

Excitability scores were recorded weekly. They were measured as described by Voisnet *et al*. (1997), Kannan *et al.* (2002), and Maria *et al*. (2004). While weighing, a score of one to four was allocated to each rabbit by a single observer; a higher score representing a greater level of excitability. A score of one was allocated to a rabbit that was calm, and made little movement during the handling. Two was allocated to a rabbit that occasionally shook itself in an attempt to escape, while three was assigned to a rabbit that continuously attempted to free itself. A score of four was given to those that struggled violently throughout the entire weighing period.

Statistical Analysis

All the data obtained were subjected to analysis of variance and where significant difference exists, the Means were separated using Duncan multiple test. Data were expressed as mean of standard error of mean. Values of $P < 0.05$ were considered significant.

RESULTS

The recorded PCV and haemoglobin concentration in rabbits in diet 1 was significantly ($P < 0.001$) lower than rabbits in the remaining 3 diets, similarly total RBC of 5.68 ± 0.18 and 5.43 ± in diet 3 and 4 respectively was significantly ($P < 0.05$) higher than rabbit on diet 1 and 2. Total WBC and differential has no specific pattern and they are not significantly ($P > 0.05$) different.

The percentage haemolysis recorded at NaCl concentration of 0.85 % was lowest in all the groups; the median and maximum corpuscular fragility occurred at 0.45 % and 0.1 % NaCl concentration. The percentage haemolysis recorded at 0.3 % to 0.8 % was significantly ($P < 0.05$) higher in rabbits in diet 1 compared to the remaining 3 diets (Figure 1). The results of the excitability in experimental and control rabbits are shown in Figure 2. An excitability score of 4 was recorded in 70.00 ± 5.50 % in rabbit

Table 1: Composition of Experimental Diets

Ingredients	Dietary Treatment			
	T1	T2	T3	T4
Maize (kg)	11.75	11.75	11.75	11.75
Full fat soya beans (kg)	6.38	6.38	6.38	6.38
Rice offal (kg)	4.50	4.50	4.50	4.50
Brewer dry grain (kg)	1.50	1.50	1.50	1.50
Bone Meal (Kg)	0.75	0.75	0.75	0.75
Common Salt (g)	02.5	02.5	02.5	02.5
Mineral and Vitamin (g)	62.5	62.5	62.5	62.5
Additive (g)	0	62.5	125	187.5
Total	25.00	25.00	25.00	25.00

Table 2: Haematological Parameters of Rabbit Fed Graded level of *Hibiscus sabdariffa*

	Dietary Treatments			
Haematological Parameters	T1	T2	T3	T4
Packed Cell Volume	35.33 ± 0.88	42.00 ± 0.58	38.00 ± 1.00	38.67 ± 1.76
Haemoglobin Concentration	11.77 ± 0.30	14.00 ± 0.19	12.67 ± 0.34	12.89 ± 0.59
Total Erythrocyte	3.82 ± 0.54	4.67 ± 0.36	5.68 ± 0.18	5.43 ± 0.48
Total leukocyte	2.13 ± 0.33	2.40 ± 0.61	2.30 ± 0.30	2.73 ± 0.33
Lymphocyte	42.00 ± 10.15	34.33 ± 2.91	43.50 ± 13.50	38.33 ± 0.67
Monocyte	4.00 ± 1.15	5.33 ± 0.33	5.00 ± 1.00	4.33 ± 0.88
Neutrophils	52.33 ± 8.84	57.00 ± 3.61	46.50 ± 15.50	54.00 ± 2.52
Basophils	0.67 ± 0.33	2.00 ± 0.58	2.50 ± 1.50	2.00 ± 1.15
Eosinophils	1.0 ± 0.58	1.67 ± 0.33	2.00 ± 1.00	1.00 ± 1.00

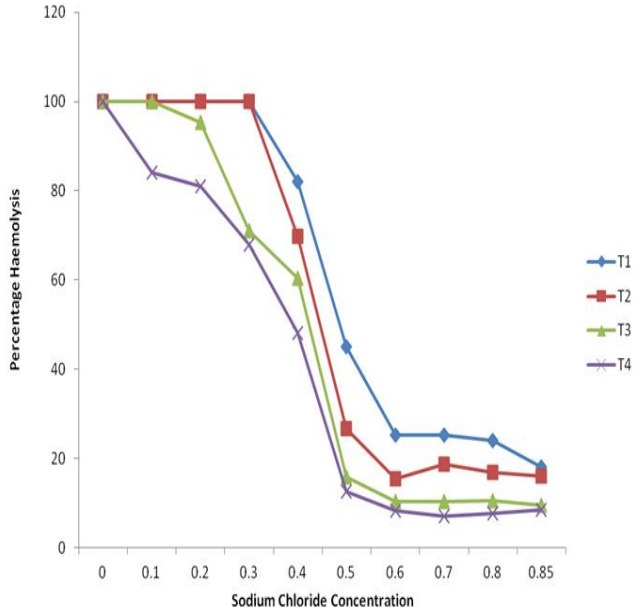

Figure 1: Erythrocyte Osmotic Fragility of Rabbit Fed Graded level of *Hibiscus sabdariffa*

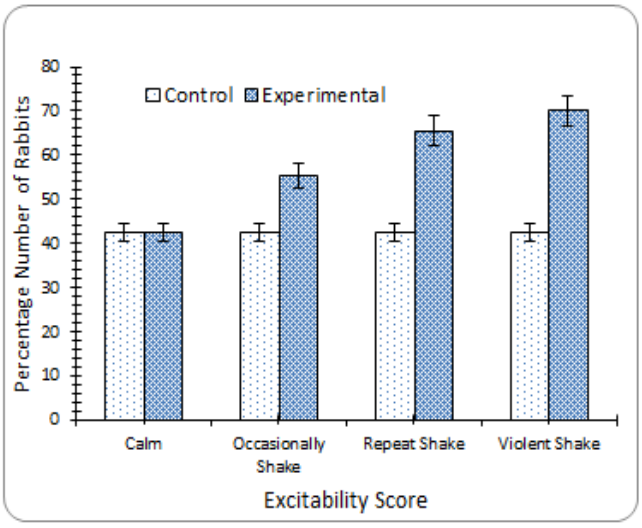

Figure 2: Excitability Score of Rabbit Fed Graded level of *Hibiscus sabdariffa*

on diet 4, while rabbit on diet 1 and 2 had a lower value which was significantly ($P < 0.05$) lower than rabbits on diets 3 and 4 with a value of 65.5 ± 5.0 % and 70.00 ± 5.50 % respectively.

DISCUSSION

In recent time, researchers has focused their attention on the protective biochemical functions of naturally occurring antioxidants in biological systems (Okasha *et al*., 2008; Bako *et al*., 2009 Ahur *et al*., 2010) and most of this plants contain phenolic compounds widely distributed in them and these were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living systems (Stanner *et al*., 2004; Olatunji *et al*., 2006). The non-significant in total WBC and differential leukocyte value obtained in this study attributed to the fact that the experimental diets are in no way inferior to other conventional feeds of rabbits and don't pose any nutritional stress to the animals. The obtained values of PCV, Hb and concentration and total RBC in rabbits on diet T_3 and 4 could be attributed to the fact that higher concentration of the HSC possibly supports haematopoesis and HSC has been documented to induce renal secretion of erythropoietin which is needed for proliferative and maturative stages of the erythroblast (pluripotent stem cells) involved in cell formation (Kaur and Kapoor, 2005). The normal function of the erythrocyte is largely hinged on the maintenance of the integrity of its membrane. The lysis of the erythrocyte membrane resulting in increased haemolysis as seen in rabbit on diet 1 (control) may have risen from the increased lipoperoxidative changes which has been documented to cause increase haemolysis (Ambali *et al*., 2010) and this might have led to less haemolysis seen in rabbit on diet 4 in this study.

In contrast the lower percentage of haemolysis recorded in rabbits on experimental diets (2, 3, 4) increases as the percentage of inclusion decreases could possibly be due to the fact that HSC possess and antioxidant property that consolidates the integrity of erythrocyte membranes of and, therefore reduces their oxidative damage. The destruction of erythrocytes observed in this study as evidenced by increase in haemolysis, which act as powerful free radical generators when found as free ions in high concentration (Adenkola, 2010). The extract of HSC has been demonstrated to protect cells against

oxidative stress in rats (Wang *et al*., 2000) and that the antioxidant effect of HSC may be due to the fact that the calyces of the plant contain ascorbic acid and tocopherol as revealed by the preliminary phytochemical screening (Ali *et al.*, 2005; Mohammed *et al*., 2007; Bako *et al*., 2009). This effect seen in this study may be attributed to the possible antioxidant effect of HSC.

Excitability of animals depends on their temperament and temperament in animals is a trait that seems to be stable over time. HSC has been documented to contain vitamin C (Wang *et al*., 2000; Essas *et al*., 2006) and other antioxidant principles (Tee *et al*., 2002; Ologundudu *et al*., 2009). Vitamin C as an antioxidant has been documented to increase excitability scores in animal (Ayo *et al.*, 2006) possibly because it plays a significant role in the synthesis of vitaminergic neurotransmitters in the brain. The findings in this study demonstrated the ability of HSC content of the feed to activate the nervous system especially in rabbit fed higher concentration in their feed. This could possibly explain the higher excitability score seen in rabbits fed higher concentration of HSC in their feed as additives.

In conclusion this study demonstrated for the first time that using of HSC in feed will improves haematological parameters, brain mood and function as well as maintaining erythrocyte membrane integrity.

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Estimation of Plasma Arginine Vasopressin Concentration Using Thirst Perception and Plasma Osmolality Values

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Summary: In human, thirst and antidiuretic hormone (ADH) are controlled by similar sensitive osmoregulatory mechanisms such that above a certain osmotic threshold (280-288 mOsm/kg H_20) there is a linear relationship between the increase in plasma osmolality and increase in ADH and thirst. The purpose of this study was to estimate plasma arginine vasopressin (P_{AVP}) using thirst perception (TP) and plasma osmolality (P_{OSM}) values before and at 60 minutes in control or euhydrate (group A, 0.0 ml/kg body weight of distilled water), hydrated (group B, 7.1ml/kg body weight of distilled water) and dehydrated (group C, 0.0 ml/kg body weight of distilled water) subjects. A total of twenty five (25) subjects between the ages of 18 and 30 years were used for the study. Calculated P_{OSM} and TP values were used to estimate the P_{AVP} concentration. Data were presented as Mean ± SEM. Analyses of results were done using ANOVA and Student *t*-test. The estimated values of P_{AVP} using TP and P_{OSM} respectively at baseline levels were similar in euhydrate (2.22±2.00 vs 2.40±2.10 pg/ml), hydrate (2.22±1.34 vs 2.40±1.72 pg/ml) and in dehydrate (7.05±1.70 vs 6.92±1.94 pg/ml). Sixty minutes later, the values remained similar in euhydrate (3.29±2.40 vs 4.16±2.10 pg/ml), hydrate (1.92±1.60 vs 1.79±1.25 pg/ml) and in dehydrate (8.40±1.40 vs 9.20±1.50 pg/ml). The results show that there was a positive relationship between P_{AVP} calculated from TP and P_{OSM} values. We therefore concluded that plasma arginine vasopressin concentration may be estimated using thirst perception and/or plasma osmolality values. Estimation of P_{AVP} using plasma osmolar changes affected by glucose and urea may be inappropriate.

Keywords*:* Arginine vasopressin, Thirst perception, Plasma Osmolality.

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INTRODUCTION

Arginine vasopressin (AVP) also referred to as antidiuretic hormone (ADH) is a nine amino acid peptide with a 6-member disulphide ring**.** It is structurally related to oxytocin but differ in two amino acids. It is synthesized majorly by the supraoptic nuclei and in a minute quantity by the paraventricular nuclei of the hypothalamus and eventually stored in the posterior pituitary. It has a very powerful antidiuretic action (Tijssen, 1985) and is the principal hormone involved in the conservation of water by the kidney. Its main effect is found in the collecting ducts where it causes insertion of aquaporin 2 (AQP2) water channels on the apical membrane (Nielson et al., 1993; Nielson et al., 1995; Fushimi et al., 1997) thus increasing permeability to water. It has been shown that AVP is released by both osmotic and non-osmotic stimuli (Clarke et. al., 1979; Malvin, 1971). It is also a potent pressor agent, through the activation of the vascular V1-receptors. Arginine Vasopressin secretion is regulated mainly by changes in the osmolality of blood and in extracellular fluid volume.

Disturbances of the secretion or function of vasopressin can cause profound clinical abnormalities in sodium and water homeostasis (Mckenna and Thompson, 1998). Serum AVP measurement is used clinically for studies involving diabetes insipidus, syndrome of inappropriate ADH secretion (SIADH), ectopic AVP production and psychogenic water intoxication (Haynes, 1958). These measurements are usually very expensive.

Robertson (1984) assessed the effects of osmotic stimuli on thirst mechanism and vasopressin secretion at various times during infusion of hypertonic sodium chloride solution in healthy adults. The result showed that the function, plasma arginine vasopressin concentration, P_{AVP} = 1.48 (P_{OSM} - 284.7) and thirst perception, TP = 9.06 (P_{OSM} - 293.5). According to his analysis, the osmotic threshold for the onset of thirst was 293.5mOsm/kg H_2O, which is approximately 10mOsm/kg H_2O above the osmotic threshold of vasopressin release. This analysis suggests that thirst rarely occurs in situations where plasma osmolality lies within the normal physiological range.

The major stimulus for thirst is an increase in plasma osmolality, which is regulated primarily by changes in the concentration of electrolytes in blood, measured as the osmolality of blood plasma. Oral fluid loads and dehydration show a consistent thirst perception in man (Obika et. al., 2009). In 2008, Igbokwe and Obika showed that thirst perception as

recorded with the VAS can be related to the concentration of plasma AVP in normal subjects. More recently, Amabebe et al. (2012) measured plasma AVP using AVP ELIZA and showed clearly that there is a relationship between plasma AVP concentration and thirst perception in man. Thus, thirst and ADH mechanism operate in such a way that the regulation of body fluid volume is dependent on the activity of the hormone ADH as well as an intact thirst mechanism.

A number of groups have employed the simple technique, the visual analogue scale, devised by Thompson et al. (1986) to measure thirst and showed that thirst perception does not change within the physiological range of plasma osmolality and that the thirst rating so obtained correlate closely with plasma osmolality. Thirst responses defined by this method are highly reproducible within an individual (Thompson et. al., 1991) and correlates well with the subsequent volume of water drunk (Thompson et. al., 1986; Obika et. al., 2009). Tiplady et al. (1998) established the validity and sensitivity of the visual analogue scale in healthy young and old subjects. The pattern of result obtained did not indicate any marked differences between the age groups in the use of VAS. The VAS, though, might be susceptible to a variety of personal and cultural influences; the method provides the best available description of the function of the thirst mechanism so far (Robertson, 1984).

The aim of this study was therefore to estimate plasma arginine vasopressin using TP and P_{OSM} values in an effort to ascertain the validity of the relationship between thirst perception and estimated plasma osmolality in normal subjects in three different states of hydration.

MATERIALS AND METHODS

SUBJECTS.

A total of twenty five (25) apparently normal subjects between the ages of 18 and 30 years were used for this study. Subjects were adequately informed of the experimental procedure and only those who gave their consent were enrolled. Exclusion criteria for this study were any history of diabetes and cardiovascular diseases. The subjects were divided into three groups: A, B and C.

Group A: This consists of the control or euhydrate subjects who did not receive any form of treatment. Anthropometric data were recorded, and they remained in the laboratory for the 60 minutes duration of the experiment.

Group B: This consists of hydrate subjects. On the day of the experiment, enrolled subjects entered the laboratory and their anthropometric data were obtained and were thereafter given 7.1ml/kg water orally. They remained in the laboratory for the next 60 minutes.

Group C: This consists of dehydrate subjects who voluntarily went on 18-hour water deprivation. Prior to the day of the experiment, subjects were instructed not to drink water or any other form of fluid after 3.00pm till after the experiment the next day. They however continued to void and discard urine until 9.00pm. Thereafter and up until 9.00am on the next day (the day of the experiment), all urine was voided into a container provided. Only subjects who had a 12-hour urine volume of about 400ml were assumed to have complied with the dehydration procedure and therefore were included in this group. Anthropometric data were recorded and they remained in the laboratory for 60 minutes.

Collection of 12-hour urine sample. Prior to the day of the experiment, all the subjects in the three groups were asked to collect their 12-hour (from 9:00pm until 9:00am on the day of the experiment) urine sample into a container. Thus when they entered the laboratory by 9:00am, they were asked to empty the content of their bladder to complete the 12-hour urine sample collection.

Thirst perception rating. Thirst perception rating (cm) was obtained using the Visual Analogue Scale, VAS (Thompson et al., 1991). The VAS is an uncalibrated 10cm vertical line, with the top and base representing **"very thirsty"** and **"not thirst"** respectively.

All the subjects were educated on how to use the VAS to estimate their level of thirst perception (TP). They were then asked to mark on the line rating scale in response to the question "**How thirsty are you NOW**?''. The reading obtained from the point of not thirsty to the point marked by the subject is a subjective record of the thirst rating measured in centimeters at that point of time. There was a separate sheet of paper for each subject to rate their thirst perception. Anthropometric data such as height, ht (m) and weight, wt (kg) were recorded using measuring rule and weighing scale respectively. Body mass index (BMI) was thereafter calculated from the formula,

$$BMI = \frac{\text{Wt(kg)}}{Ht^2(m^2)}$$

Baseline (resting) blood pressures (BP, mmHg) were measured with subjects in the sitting position and after 15minutes of rest in the laboratory at room temperature. Three basal readings were obtained by indirect auscultatory method using sphygmom-anometer and stethoscope on each subject at 3-minutes interval. The mean of these readings was recorded as normal BP.

Sample collection and Analysis: Urine and blood samples as well as other data were collected when the subjects entered the laboratory. Then, 60min later samples were again collected. All urine samples were collected into a plain container to determine the volume and/or specific gravity, while all blood samples were collected and gently transferred (to avoid lysis) into the Lithium Heparin sample bottles for analysis of sodium and potassium concentrations after centrifugation; and Fluoride Oxalate sample bottles for analysis of glucose and urea concentrations respectively. All plasma, serum and urine samples were analyzed for sodium (Na^+), potassium (K^+), Glucose (Glu) and Urea (Ur) concentrations at the Chemical Pathology Laboratory of the University Benin Teaching Hospital, using standard procedures. Packed cell volume, PCV, urine volume (V), and specific gravity (SG) of urine were determined in the Physiology Laboratory of the University of Benin. PCV was determined using heparinised capillary tube, a centrifuge and haematocrit reader, while V and SG were determined using measuring cylinder and Urinalysis reagent strips respectively.

Calculations: Plasma arginine vasopressin (P_{AVP}) concentration was calculated using TP and P_{OSM} with the following equations:

Plasma Arginine Vasopressin, P_{AVP}, calculated with the formulae:

$$P_{AVP} = \frac{T(CM)-1.2}{0.75}$$ (Igbokwe and Obika, 2008) … (1)

and,

$$P_{AVP} = 0.43 \times (P_{OSM} - 284.3)$$ (Thompson et al., 1986) (2)

Plasma osmolality was obtained indirectly from thirst perception values using the equations:

$$P_{OSM} = 10TP/3 + 281$$ (Thompson et al., 1986)… (3)

and from,

$$P_{OSM} = 2Na + \frac{Glucose}{18} + \frac{BUN}{2.8}$$ (Purssell et al., 2001) (4)

Statistical Analyses

Data were presented as Mean ± SEM. Analyses of results were done using ANOVA and Student *t*-test. Correlation between calculated P_{AVP} using TP and P_{OSM} were determined. P values less than 0.05 were considered statistically significant.

RESULTS

This study compared calculated P_{AVP} using TP and P_{OSM} in euhydrate (group A), hydrate (group B) and dehydrate (group C) subjects at zero minute (fifteen minutes after the subjects entered the laboratory) a well as between zero minute and after 60minutes in each group A, B and C. Results in table 1 show that the parameters in all the groups were comparable to each other.

Results in table 2 show that at baseline, the PCV was higher in dehydrate group than in both euhydrate and dehydrate groups, but not statistically significant. However, after 60 minutes PCV was significantly higher in group C compared to groups A and B. When compared to the baseline values, hydration (group B) caused a significant fall in PCV after 60 minutes, while in euhydrate and dehydrate subjects, the PCV remained unchanged. The table also shows that at baseline, specific gravity of urine (SG) was significantly higher in group C subjects than in both groups A and B. In addition, after 60 minutes, it was significantly higher when compared to the 60 minutes

Table 1: Anthropometric data in Euhydrate, Dehydrate and Hydrate subjects at zero min (Baseline). Values are Mean ± SEM.

Group	Age, yrs.	Ht, m	Wt, kg	BMI, kg/m^2	SBP, mmHg	DBP, mmHg
A (N = 5)	21.6±0.81	1.65± 0.04	65.9± 3.6	24.40± 1.96	112.3±3.5	69.6± 3.2
B (N = 10)	20.8±0.47	1.82± 0.09	61.9± 3.0	20.98± 0.72	112.8± 2.0	73.2± 2.1
C (N = 10)	22.6±0.87	1.69± 0.03	69.2± 3.2	21.76±0.99	111.2± 2.4	76.8± 2.4

Table 2: PCV, Specific Gravity and 12-h urine volume in Euhydrate, Dehydrate and Hydrate subjects at zero minute (Baseline) and at 60 minutes Values are Mean ± SEM.

Group		PCV, %	SG (urine)	UV, ml
A (N = 5)	At Baseline	43.8 ± 3.8	1.014 ± 0.001	692 ± 10.2
	At 60 minutes	41.0 ± 3.7	1.016 ± 0.002$^+$	-
B (N = 10)	At Baseline	42.7 ± 1.8	1.009 ± 0.001	652 ± 28.6
	At 60 minutes	40.8 ± 1.8 $^+$	1.005 ± 0.001$^+$	-
C (N = 10)	At Baseline	44.4 ± 2.2	1.021 ± 0.001*	327 ± 20.7*
	At 60 minutes	45.9 ± 2.2 $^\#$	1.022 ± 0.001$^+$	-

**= p<0.05 between Dehydrate, Hydrate and Euhydrate subjects at baseline.*

#= p<0.05 between Dehydrate, Hydrate and Euhydrate subjects at 60 minutes.

+= p<0.05 between baseline and after 60 minutes values in each group (A, B and C).

Table 3: Calculated P_{AVP} using Thirst perception and Plasma Osomolality values in Euhydrate, Dehydrate and Hydrate subjects at zero minute (baeline) and at 60 minutes. Values are Mean ± SEM

Group		Thirst perception, (TP) cm.	P_{AVP} (pg/ml) calculated from TP (Igbokwe & Obika, 2008).	Plasma osmolality, (P_{osm}) mOsm/kg (Thompson et. al., 1986).	P_{AVP} (pg/ml) calculated from P_{osm} (Thompson et. al., 1986).	Plasma osmolality (P_{osm}) mOsm/kg (Purssell et al., 2001).	P_{AVP} (pg/ml) calculated from Posm (Purssell et al., 2001).
A(N = 5)	0 min.	3.06 ± 1.4	2.27±2.00	291.06±4.8	2.91±2.10	274.22±1.93	-4.34±1.9
	60 min.	3.68±1.80	3.29±2.40	293.98±6.1^{+}	4.16±2.60^{+}	277.84±0.98^{+}	-2.87±0.43
B (N = 10)	0 min.	2.80 ±0.9	2.22±1.34	290.33±2.9	2.40±1.72	286.16±3.66	0.80±1.6
	60 min.	2.64±1.20	1.92±1.60	289.74±4.0^{+}	1.79±1.25	278.34±2.21^{+}	-2.60±0.95
C (N = 10)	0 min.	6.43 ±1.3*	7.05±1.7*	300.46±4.5	6.92±1.94*	279.79±5.0	-1.96±2.06
	60 min.	7.50±1.05*$^{+}$	8.4±1.40^{+}	305.98±3.5^{+}	9.20±1.50^{+}	283.53±4.15^{+}	-0.33±1.79

* *= p<0.05 between Dehydrate, Hydrate and Euhydrate subjects at baseline*

$^{+}$*= p<0.05 between baseline and after 60 minutes values in each group, (A, and C).*

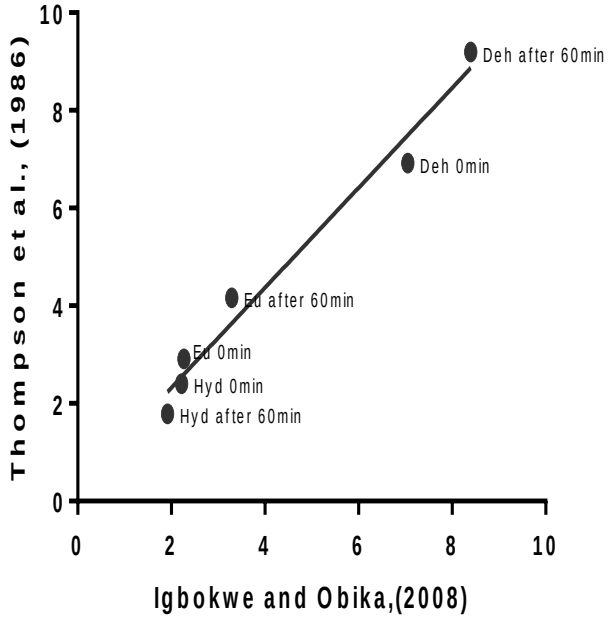

Figure 1. Relationship between the calculated P_{AVP} using the formula of Igbokwe and Obika (2008) and that of Thompson et. al. (1986).

values in groups A and B subjects. Values at baseline and at 60 minutes in groups A and B remained similar. Furthermore, the 12-h urine volume (UV) was significantly lower in group C subjects when compared the values in both group A and B subjects.

Results in table 3 show that TP was significantly higher in group C subjects than in both group A and B subjects at baseline. After 60 minutes, there was an increase in TP in group A and C subjects but this was significantly higher only in group C while in group B, it was lower but not significant.

P_{OSM} (calculated from the formula of Thompson et al., 1986) was not significantly different between the three groups at baseline. It was however significantly higher after 60 minutes in both A and C groups and significantly lower in group B compared to baseline.

P_{AVP} calculated from TP (Igbokwe and Obika, 2008) and that calculated from P_{OSM} (Thompson et al., 1986) were significantly higher in C group at baseline. After 60 minutes, P_{AVP} was significantly higher in group A (from only the formula of Thompson et al., 1986) and in group C from both formulae, but there was no significant difference in group A from the formula of Igbokwe and Obika, (2008) and in group B from both formulae.

When the formula of Pursell et al., (2001) was applied in the calculations of both P_{OSM} and P_{AVP}, there was no significant difference in the three groups at baseline and the results were not consistent. After 60 minutes, P_{OSM} of subjects in groups A and C were significantly higher while that of the subjects in group B was significantly lower. There was no significant difference in P_{AVP} of the three groups after 60 minutes. There was a positive and significant ($p<0.01$) linear relationship between between the calculated P_{AVP} using the formula of Igbokwe and Obika (2008) and that of Thompson et. al. (1986) as shown in figure 1.

DISCUSSION

These experiments were designed to estimate P_{AVP} using TP and P_{OSM} values. Therefore we set out to develop an alternative method to estimate plasma arginine vasopressin concentration without measuring it or measuring plasma osmolaity.

Edwards (1971) reported that the normal P_{AVP} concentration measured by immunoassay is about 1-5 pg/ml. The Cayman Chemical Company (2011), using the arginine vasopressin Enzyme immunoassay (EIA) kit showed that normal levels of AVP in serum are between 0.4 and 5.2pg/ml. Kamath (2010) had shown that in healthy adults who had no fluid restrictions and had a normal activity level, the normal plasma concentration of ADH is between 0.35-1.94 ng/l (0.32-1.80pmol/l). Our result agrees with these findings, where P_{AVP} in euhydrate and hydrate subjects at baseline calculated from the

formula of Thompson et al. (1986) and that of Igbokwe and Obika (2008) were similar. The result as obtained with the formula of Purssell et al. (2001) were however negative and not within the same range, except for the hydrate subjects at zero minute. The probable explanation for this is that the equation involves substances that affect osmolality without affecting other substances that influence plasma osmolality like sodium and urea concentrations. And since it has been established that sodium is a major determinant of serum osmolality, it implies that P_{AVP} concentration will be indirectly affected.

Nevertheless the calculated P_{AVP} from TP (Igbokwe and Obika, 2008) and P_{OSM} (Thompson et al., 1986) were similar in the three groups (euhydrate, hydrate and dehydrate subjects), even when the calculated P_{AVP} in euhydrate and dehydrate subjects increased after 60 minutes and decreased in hydrate subjects after 60 minute. This implies that application of the stimulus (dehydration) increased both P_{AVP} concentration and TP, while removal of the stimulus (hydration) suppresses them. When P_{AVP} concentration was calculated from plasma osmolality using Purssell et. al. (2001) equation, the values also increased in euhydrate and dehydrate subjects while in hydrate subjects it reduced but as mentioned earlier these values were negative except for the hydrate subjects at baseline.

Karkare (2010) showed that if serum osmolality is more than 290 mOsm/kg H_2O, the ADH levels should be around 2-12pg/ml, and when it is less than 290mOsm/kg H_2O, the ADH levels should be less than 2pg/ml. Our results also agree with these findings as shown in tables 3 and 5 where the plasma osmolality calculated from Thompson et al., (1986) was 290.33mOsm/kg H_2O, and 289.8mOsm/kg H_2O, with the corresponding ADH values of 2.36pg/ml and 1.79pg/ml respectively.

However, when the formula of Purssell et al., (2001) was used, the values obtained were not within the physiological range of plasma ADH concentration, except for hydrate subjects at zero minute. This suggests that this formula may not be ideal for the estimation of P_{AVP} concentration in certain situations.

The baseline TP reported in these studies are similar to earlier reports of Obika et al., (1996) in young healthy non dehydrated subjects. Obika et al. (2009) also showed that after a period of dehydration, normal subjects showed an increase in TP. The results reported here agree with these earlier reports.

The subjects who dehydrated for 18hours (group C) had a decrease in urine volume and an increase in specific gravity indicating that there was increase in urine osmolality and consequently increase in P_{AVP} secretion and TP. Furthermore, previous studies by Thompson et al. (1986), Takamata et al. (1994) and Baylis and Robertson (1980) showed that the increase in TP during dehydration correlates positively with increase in packed cell volume. Our work agrees with these findings where PCV was higher in dehydrate subjects compared to both euhydrate and hydrate subjects

Amabebe et al. (2012) using the arginine vasopressin Enzyme Immunoassay (EIA) to determine P_{AVP} concentration showed that a linear relationship exists between measured P_{AVP} and TP (cm) and from the present study, linear relationship also exists between calculated P_{AVP} and TP (cm) in that after a period of dehydration, P_{AVP} increased concomitantly with TP and decreases as well with hydration.

The validity of measurements of subjective rating of thirst has been previously reported as follows: thirst correlates positively with P_{OSM} (Baylis and Robertson, 1980). Thirst ratings using VAS was also found to correlate positively with P_{OSM} (Thompson et al., 1986; Takamata et al., 1994). From our study, it was shown that TP and P_{OSM} were higher in dehydrate subjects than in both euhydrate and hydrate subjects, also suggesting that after dehydration, there is a concomitant increase in TP and $P_{OSM.}$ and vice versa. These findings thus agree with the earlier reports of Baylis and Robertson (1980), that above certain osmotic threshold (280-288 mOsm/kg H_2O), there is a linear relationship between increase in P_{OSM} and increase in ADH and thirst.

In summary, this study shows that there was a positive relationship between P_{AVP} calculated from TP (cm) and that calculated from P_{OSM}; between TP (cm) and P_{OSM}; between calculated P_{AVP} and TP (cm) as well as between calculated P_{AVP} and P_{OSM}. This work therefore was found to validate the findings and equations put forward by Igbokwe and Obika, (2008) and Thompson et al. (1986), and further established that there is a linear relationship between plasma arginine vasopressin, P_{OSM} and thirst.

We can therefore conclude that plasma arginine vasopressin concentration may be estimated using thirst perception and/or plasma osmolality values. However, caution should be taken when other factors that may not directly affect osmolar changes are involved.

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Lactational Vitamin E Protects Against the Histotoxic Effects of Systemically Administered Vanadium in Neonatal Rats

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Summary: The work investigated the protective role of lactational vitamin E administration on vanadium-induced histotoxicity. Three groups of Wistar rats, with each group comprising of two dams and their pups, were used in this study. Group I pups were administered intraperitoneal injection of sterile water at volumes corresponding to the dose rate of the vanadium (sodium metavanadate) treated group from postnatal day (PND) 1-14 while those in Group II were administered intraperitoneal injection of 3mg/kg vanadium from PND 1-14. Group III pups were administered intraperitoneal injection of 3mg/kg vanadium while the dam received oral vitamin E (500mg) concurrently every 72hours. The results showed that group II pups exhibited histopathological changes which included seminiferous tubule disruption of the testes characterised by vacuolar degeneration and coagulative necrosis of spermatogonia and Sertoli cells with reduction in mitosis, and areas of interstitial thickening with fibroblast proliferation. In addition, the lungs showed disruption of the bronchiolar wall and denudation of the bronchiolar respiratory epithelium while the liver showed hydropic degeneration and coagulative necrosis of the centrilobular hepatocytes. These histotoxic changes were ameliorated in the vanadium + vitamin E group. We conclude that lactational vitamin E protects against the histotoxic effects of vanadium and could be a consideration for supplementation in the occupationally and environmentally exposed neonates. However, caution should be taken in vitamin E supplementation because there is still equivocal evidence surrounding its benefits as a supplement at the moment.

Keywords*:* Vanadium, Vitamin E, Histotoxicity, Antioxidant.

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INTRODUCTION

Vanadium is a major trace metal in fossil fuels that is used extensively in modern industries in the manufacture of jet air craft, phthalic anhydride, pesticides, sulphuric acid, photography and as a catalyst in the production of many materials (Wenning and Kirsch, 1988). Acute environmental and occupational exposure to vanadium and its compounds is however not uncommon (Hope, 1994) since it is a transition metal widely distributed in nature and extensively used in heavy industry (Ray et al., 2006). Sources of occupational exposure include combustion of vanadium-rich fuels, processing and refining of vanadium ores and sludges, manufacturing of vanadium-containing products, and by handling of catalysts in the chemical industry (Plunkett, 1987). With inhalation being the major route of environmental exposure (Barceloux, 1999), combustion of fossil fuels provides a significant environmental source of vanadium. The increase in environmental levels of vanadium has raised concern over the release of the element into the atmosphere from anthropogenic sources (Hope, 1994), and recently from massive oil burning as seen in Arabian Gulf (Haider *et al*, 1998), the Niger-Delta region of Nigeria (Igado *et al*., 2008), and the Gulf of Mexico (Olopade and Connor, 2011). The general population is also increasingly exposed to this metal mostly as a result of the increased utilization of vanadium containing petroleum fuel (Byczkowski and Kulkarni, 1996).

A variety of toxic effects are exerted by vanadium compounds and these effects are dependent on the oxidation state and circulating levels of vanadium. However Sanchez *et al.* in 1998 and Soares *et al.* in 2008 showed that V^{5+}(oxidative state) seems to be more toxic than V^{4+} and V^{3+}. The most affected organs, as documented by histopathological alterations, were the liver and kidney (Valko *et al.*, 2005) and intraperitoneal injections of rats with orthovanadate induced nephrotoxicity (Ciranni *et al*, 1995). The reproductive and developmental functions of rats have also been well established to be affected by vanadium (Morgan, 2003).

Vanadium, with an atomic number of 23 on the periodic table; is a transition metal element with a complex chemistry as it forms polymers frequently and can occur in various oxidative states (Nechay, 1984). Vanadium participates in reactions involving formation of reactive oxygen species and free radicals (Crans *et al*, 2004) so it is therefore not surprising that antioxidants offer protection from vanadium toxicity (Olopade et al., 2011a).

Vitamin E, a member of the fat-soluble vitamins, prevents oxidative stress by working together with a group of nutrients that prevent oxygen molecules from becoming too reactive. This group of nutrients includes vitamin C, glutathione, selenium, and vitamin B3 (George, 2010). It scavenges peroxyl radicals and binds with them to form a tocopheryl radical which can then be reduced by a hydrogen donor to its reduced state (Traber *et al*, 2011). Because vitamin E is fat soluble, it is incorporated into cell membranes and protects them from oxidative damage.

This work focused on the protective role of vitamin E on the vanadium induced histopathological changes in the liver, lungs, and testes of neonatal Wistar rats.

MATERIALS AND METHODS

Animals

Six adult female Wistar rats were housed in the experimental animal house of the Neuroscience Unit of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. They were acclimatised for a period of two weeks after which they were bred. The animals were fed with pelleted rat feed with water supplied *ad libitum*. The University of Ibadan Ethical Guideline on Use of Animals in Research was followed in this study.

Study design

There were three groups, each comprising2 dams and their pups. For each group, 6 pups were randomly selected (n=6)

Group I: Pups injected with sterile water once daily intraperitoneally (IP) from postnatal day 1-14 (PND1-14).

Group II: Pups were administered sodium metavanadate, 3mg/kg body weight once daily IP from PND 1-14 based on the work of Todoriche*t al* 2011.

Group III: Pups were administered sodium metavanadate 3mg/kg body weight IP from PND 1-14; the dams (n=2) were concurrently administered oral vitamin E (500mg) every 72 hours to deliver it to the pups via lactation (Martin and Hurley, 1977).

Preparation of tissues

The pups were anaesthetised with ketamine (100mg/kg) IP on PND 15. They were then quickly decapitated and dissected on ice. The lungs, liver, and testes were harvested. The testes were immediately fixed in Bouin's fluid while the lungs and liver were fixed in 10% formalin for 72hours.

Histopathology of tissues

Samples of harvested lungs, testes and liver were thoroughly dehydrated by passing them through graded solutions of alcohol (30%, 50%, 70% and 100%) after which they were cleared in two changes of xylene at an interval of one hour each and impregnated with three changes of paraffin wax for an hour each and finally embedded in fresh molten wax. Sections of 5µm thickness of each organ were prepared and stained with haematoxylin and eosin for examination under a light microscope.

RESULTS

Histopathology of the testes

In group II (vanadium only group), there was disruption of seminiferous tubules characterised by vacuolar degeneration and coagulative necrosis of spermatogonia and Sertoli cells with reduction in mitosis, and areas of interstitial thickening with fibroblast proliferation. These were absent in the vanadium+vitamin E and control groups (Fig.1)

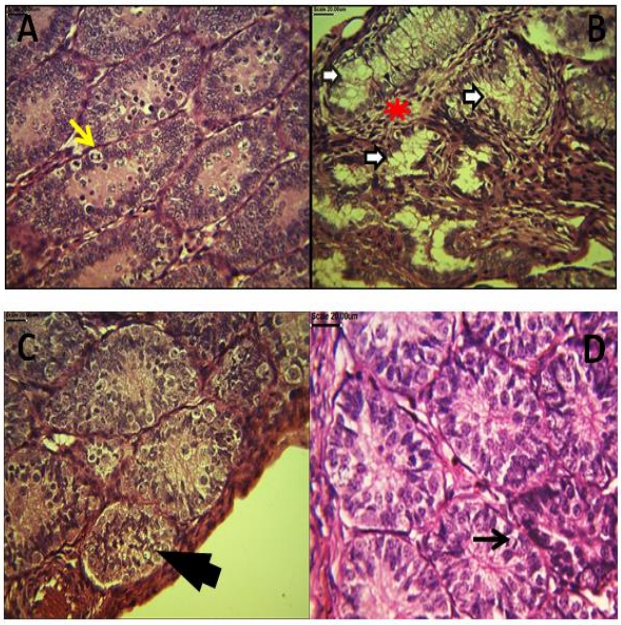

Figure 1: Transverse section of the seminiferous tubules (H&E, x400): A- control: yellow arrow showing a dividing cell; B and C: vanadium only; white arrows pointing to seminiferous tubules with severe loss of the germinal epithelium due to vacuolar degeneration and necrosis, red star indicating area of interstitial thickening; black arrow pointing to a tubule with necrotic germinal cells; D: vanadium + vitamin E; black arrow showing a dividing cell.

Histopathology of the lungs

There was disruption of the bronchiolar wall and denudation of the bronchiolar respiratory epithelium in the lungs of the vanadium only group; this was in contrast with the well-defined smooth muscle layer of the bronchiolar walls of the vanadium+ vitamin E and control groups. (Fig.2).

Histopathology of the liver

There was hydropic degeneration and coagulative necrosis of the centrilobular hepatocytes in the vanadium only group. This was absent in the vanadium + vitamin E and control groups. (Fig.3)

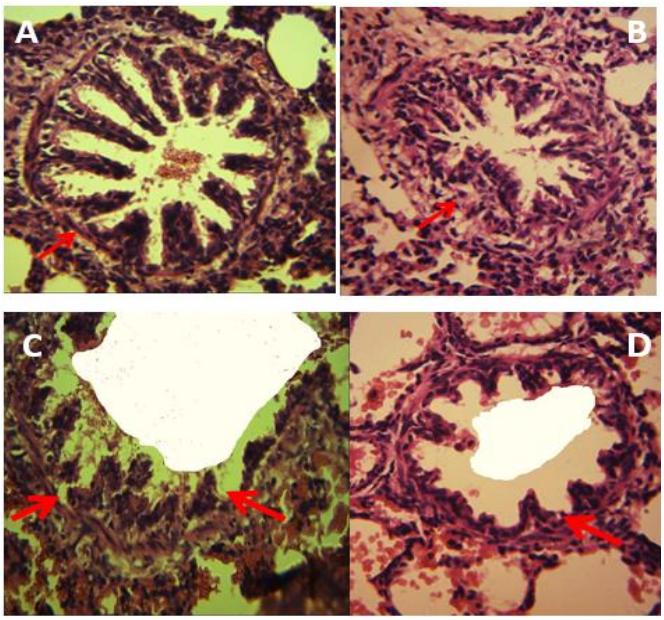

Figure 2: Bronchioles, Cross Section, H&E x400: A-control; the bronchiolar architecture is well defined with the arrow pointing to the smooth muscle of the laminar propria. B-vanadium only; there is disruption of the bronchiolar smooth muscle/connective tissue (red arrow). C-vanadium only;the red arrows are pointing to areas of epithelia sloughing.D-vanadium+ vitamin E; note the intact smooth muscle/connective tissue layer in the lamina propria (red arrow)

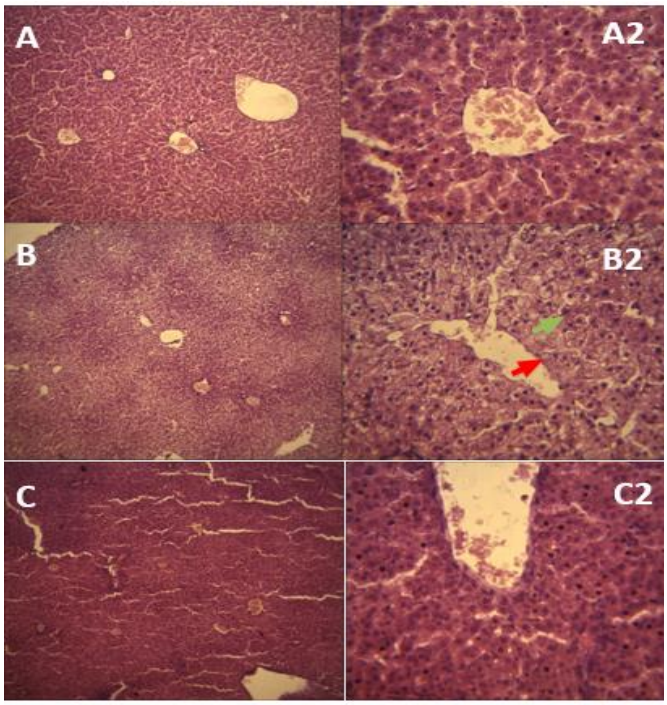

Figure 3: Liver, Transverse Section, H&EA: Control showing the normal liver architecture (x100) A2: Control, centrilobular venule (x400) B: Vanadium only. The pale areas around the central veins are areas of hydropic degeneration and coagulative necrosis (x100) B2-Vanadium only(x400).Hepatocytes of the centrilobular zone are necrotic (red arrow) with others showing hydropic degeneration (green arrow) C: Vanadium + vitamin E (x100) normal liver architecture. C2- Vanadium + vitamin E (x400) showing no visible pathology.

DISCUSSION

Vanadium induces production of reactive oxygen species which have a range of deleterious effects on the cell metabolism such as lipid peroxidation, denaturation of intracellular proteins and destruction of DNA (Shi *et al*, 1996). In this study, vanadium was shown to induce necrotic and degenerative changes in the testes, lungs and liver.

In the testes, vanadium inducednecrosis and vacuolar degeneration of the spermatogonia and Sertoli cells coupled with reduction in mitosis. Vanadium has been reported to induce G2/M phase arrest in a time- and dose-dependent manner (Zhang *et al*, 2001); this most likely explains the reduction in mitosis in the vanadium exposed testis.

In the liver, vanadium exposure resulted in marked centrilobular hepatocellular injury, characterised by hydropic degeneration and coagulation necrosis. Hepatocytes in the centrilobular zone have a high composition of cytochrome p-450 enzymes (Stalker and Hayes, 2007). These enzymes have been implicated in the production of reactive oxygen species (Halliwell and Gutteridge, 1990). Thus, the pronounced centrilobular necrosis and degeneration can be said to be expected in the vanadium exposed group.

A previous study on vanadium pentoxide showed that it induces bronchial hyper responsiveness and asthma in vanadium plant workers (Irsigler *et al*, 1999). In this current study on sodium metavanadate, the disruption of the bronchiolar smooth muscle observed might explain the asthma-like symptoms seen in people exposed to vanadium via inhalation.

In 2011, Traber *et al.* established that vitamin E binds with peroxyl radicals to form a tocopheryl radical which can then be reduced by a hydrogen donor and this antioxidative property of vitamin E is effective within a cell as vitamin E is fat soluble is easily incorporated into cell membranes. Vitamin E administration attenuated the histotoxic effects of vanadium, as shown above, on the testes, liver and lungs of the pups. This result agrees with both the earlier report by Olopade *et al* (2011b) who administered both vanadium and vitamin E via the lactation route and with the work of Uche *et al* (2008) who showed that vitamin E prevented and reversed morphophysiological deficits induced by vanadium in the testes and liver.Vitamin E via the lactation route has also been shown to protect neonatal rats from vanadium-induced reactive astrogliosis and behavioural deficits (Olopade *et al.*, 2011a).

It can be deduced from this study that lactational vitamin E administration protects against the histotoxic effects of vanadium and could be considered for supplementation in the occupationally and environmentally exposed neonates. However, caution should be taken in vitamin E supplementation because there is still equivocal evidence supporting

its benefits as a supplement at the moment (Haber, 2006; Gallo *et al*., 2010; Abner *et al,* 2011; Olopade *et al.*, 2011b)

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The Aqueous Calyx Extract of *Hibiscus sabdariffa* Lowers Blood Pressure and Heart Rate via Sympathetic Nervous System Dependent Mechanisms

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Summary: The antihypertensive effect of *Hibiscus sabdariffa* (HS) has been validated in animals and man. This study tested the hypothesis that its hypotensive effect may be sympathetically mediated. The cold pressor test (CPT) and handgrip exercise (HGE) were performed in 20 healthy subjects before and after the oral administration of 15mg/Kg HS. The blood pressure (BP) and heart rate (HR) responses were measured digitally. Mean arterial pressure (MAP; taken as representative BP) was calculated. Results are expressed as mean ±SEM. $P<0.05$ was considered significant. CPT without HS resulted in a significant rise in MAP and HR (111.1±2.1mmHg and 100.8±2.0/min) from the basal values (97.9±1.9mmHg and 87.8±2.1/min; $P<0.0001$ respectively). In the presence of HS, CPT-induced changes (ΔMAP=10.1±1.7mmHg; ΔHR= 8.4±1.0/min) were significantly reduced compared to its absence (ΔMAP= 13.2±1.2mmHg; ΔHR= 13.8±1.6/min; $P<0.0001$ respectively). The HGE done without HS also resulted in an increase in MAP and HR (116.3±2.1mmHg and 78.4±1.2/min) from the basal values (94.8±1.6mmHg and 76.1±1.0/min; $p<0.0001$ respectively). In the presence of HS the HGE-induced changes (ΔMAP= 11.5±1.0mmHg; ΔHR= 3.3±1.0/min) were significantly decreased compared to its absence (ΔMAP=21.4±1.2mmHg; ΔHR= 12.8±2.0/min; $P<0.0001$ respectively). The CPT and HGE -induced increases in BP and HR suggest Sympathetic nervous system activation. These increases were significantly dampened by HS suggesting, indirectly, that its hypotensive effect may be due to an attenuation of the discharge of the sympathetic nervous system.

Keywords*: Hibiscus sabdariffa*, Cold pesssor test, Hand grip exercise, Blood pressure, Heart rate

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INTRODUCTION

The aqueous calyx extract of *Hibiscus sabdariffa* (HS; Family: Malvaceae) is used in Nigerian folk medicine to treat hypertension (Oliver, 1960). Its folk reputation as an antihypertensive agent has been validated in hypertensive animals (Onyenekwe *et al.*, 1999; Odigie *et al.*, 2003; Mojiminiyi *et al.*, 2007; Mojiminiyi *et al.*, 2012) and man (Haji Faraji and Haji Tarkhani, 1999; Herrera-Arellano *et al.*, 2004; Herrera-Arellano *et al.*, 2007; Mckay *et al.*, 2009). The constituents of HS include protein, fat, carbohydrate and fibre (Da-Costa-Rocha et al., 2014). It is abundant in vitamin C, β-carotene, calcium and iron (Ismail et al., 2008). It also contains organic acids, anthocyanins, polysacharrides and flavonoids (Müller and Franz, 1990). Anthocyanins (e.g. cyanidin-3-sambubioside and delphindin-3-sambubioside) are believed to be the major bioactive compounds producing different antihypertensive and cardioprotective effects (Jonadet et al., 1990 and Meunier et al., 2009). Within 7 hours of post HS consumption cyanidin-3-sambubioside, delphindin-3-sambubioside and total anthocyanins appear in the urine (Frank *et al.*, 2005). HS calyces are considered to be relatively non-toxic. They have a low degree of toxicity with LD_{50} between 2,000 to 5,000mg/kg/day (Hopkins et al., 2013).

Attempts have been made to delineate its mode of action. It has been reported to have cardioprotective (Jonadet, 1990), hypocholesterolemic (Chen *et al*, 2008), antioxidant (Wang *et al*, 2000, Amin and Hamza, 2005) and diuretic effects (Mojiminiyi *et al.*, 2000; Ajay *et al.*, 2007 and Alarcon-Alonso *et al.*, 2011). In addition it has vasorelaxant (Obiefuna *et al.*, 1993; Adegunloye *et al.*, 1996) and angiotensin-converting enzyme inhibitory effects (Ojeda *et al.*, 2010). Three recent excellent reviews (Hopkins *et al.*, 2013; Da-costa-Rocha *et al.*, 2014; Guardiola and Mach, 2014) have appeared in the literature suggesting a gratifyingly heightened interest in HS.

However, the role of the sympathetic nervous system, if any, has not been widely investigated. This is in spite of its primacy in both the control of normal blood pressure (Guyenet, 2006) or its dysfunction in hypertension (Guyenet, 2006) and other cardiometabolic diseases such as diabetes, syndrome

(metabolic syndrome) X e.t.c. Several classes of cardiovascular sympathetic efferents: thermosensitive, glucosensitive and barosensitive regulate arterial blood pressure (Dempsy *et al.*, 2002; Janig, 2003; Vallbo *et al.*, 2004; Guyenet, 2006). The barosensitive sympathetic efferents control the heart and kidneys and are responsible for short-term blood pressure fluctuations (Blessing, 1997; Janig, 2003). These efferents are also likely to be the key determinants of the long-term neural control of blood pressure. This is because renin secretion, kidney tubular reabsorption and renal blood flow are all controlled by barosentitive sympathetic efferents (DiBona and Kopp, 1997)

Consequently, the present study tested the hypothesis that the hypotensive effect of HS may occur through the inhibition of the sympathetic nervous system. This was achieved by using two maneuvers, cold pressor test (CPT) and hand grip exercise (HGE) that are known to activate the sympathetic nervous system (Victor *et al.*, 1987: Delaney *et al.*, 2010 respectively).

MATERIALS AND METHODS

Plant materials

The dried red calyces of HS were purchased in Talata Mafara central market, Zamfara state, Nigeria. They were identified and a voucher specimen (voucher number PCG/UDUS/MLV 001) was deposited in the herbarium of the Department of Pharmacognosy & Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Extraction procedure

The dried red calyces of HS were pounded into fine coarse powder. The powder (500g) was dissolved in 2.7 litres of hot water (50 ^{0}C) in a conical flask and mixed thoroughly by means of a magnetic stirrer. It was left overnight and later filtered using a filter paper. It was then decanted and evaporated to dryness in a water bath at 60 ^{0}C leaving a powdery extract.

Tableting of powdery extract of HS

The powdery extract was then prepared into tablets containing 500mg of extract per tablet using the wet granulation method. Table 1 gives the batch manufacturing formular. The granules were tableted using single pouch machine type (ART 400 Eureka, Gmbh, Germany)

Ethical Clearance

Before the commencement of the study, approval was obtained from the Ethical Committee of Specialist Hospital Sokoto, Nigeria. Thereafter 20 apparently healthy human (male) volunteers were randomly selected following informed consent. They were 29.9±1.6 years old and weighed 67.3±2.7 kg respectively. They were not on any medication that affects BP and HR, nor were they consuming alcohol or caffeine-containing beverages. Also, they were not involved in strenuous exercise 24 hours before the test nor did they suffer from any cardiovascular, renal or endocrine diseases.

Cold pressor test

Hine's protocol for cold pressor test as described by Wood *et al.* (1984) was used. The nature of the test was explained to the volunteers. They were weighed using the Camry Mechanical Personal Scale (Model: BR 9012, China**)**. The subjects rested for at least 30 minutes to acclimatize before recordings were made. They were then asked to lie in the supine position in a quiet room. The blood pressure and heart rate were measured using the HuBDIC EchoMax plus BP-400 digital sphygmomanometer (HuBDIC Co. Ltd., Gyeonggi-do, Korea). These were taken as the casual BP and HR. Serial blood pressure and heart rate measurements were taken at 10-minute intervals until three almost similar readings were obtained. The last of these measurements were taken as the basal blood pressure and heart rate. The subject was then asked to immerse one hand into iced water (4^0-5^0 C) just above the wrist for 1-2 minutes (Wood *et al.*, 1984). During the period of immersion, blood pressure and heart rate readings were measured in the other arm at one minute interval. The highest of these recordings was designated as peak or ceiling blood pressure and heart rate. The subjects were allowed to rest for one hour. At the end of the rest, they were given tablets of HS orally at a dose of 15mg/kg and sat down for an hour. Thereafter the procedure was repeated.

Hand grip exercise

The BP pressure and heart rate were measured at rest in the supine position. Static exercise was performed by means of hand grip which was done by asking the subject to hold a pair of pliers (Gripp plier, India) forcefully in a sustained manner for a period of 1-2 minutes, or until fatigue was felt, and the parameters were measured again. A pair of pliers was used in this study because a handgrip dynamometer was not available. The subjects were allowed to rest for one hour. HS tablets were then given orally at a dose of 15mg/kg and the procedure repeated after an hour.

RESULTS

The Blood pressure and heart rate of the subjects before and during the cold pressor test (without HS) are presented in table 2. The peak systolic (SBP), diastolic (DBP), mean arterial blood pressure (MABP) and Heart rate (HR) obtained during the cold pressor test were significantly ($P<0.0001$) higher than the basal values.

Table 1: Showing the Batch manufacturing formular for *HS* tablets

Materials	Qtty/tablet	Qtty/300 tablets
Extract (water HS)	500mg	150g
Lactose	160mg	48g
Starch	40mg	12g
Starch (mucilage)	82.4mg	24.72g
Talc	16mg	4.0g
Magnesium stearate	1.6mg	0.48g
Total	800mg	240g

Table 2: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and during the Cold pressor test (peak) without HS. SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure, MAP= Mean Arterial Pressure, HR= Heart Rate

Parameter	Basal (N= 20)	HGE; PEAK (n=20)
SBP (mmHg)	124.9±2.2	146.8±2.7*
DBP (mmHg)	79.8±1.5	101.6±1.8*
MAP (mmHg)	94.8±1.6	116.3±2.1 *
HR (beats/min	76.1±1.0	87.4±1.2*

*= P<0.0001 vs Basal values

Table 3: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and the during the Cold pressor test (Peak) in the presence of 15mg/kg HS

Parameter	CPT (n=20)	CPT + HS (n = 20)
SBP (mmHg)	147.1±2.4	137.1±1.7*
DBP (mmHg)	93.3±2.1	84.4±1.8*
MAP (mmHg)	111.1±2.2	92.4±2.1*
HR (beats/min	100.8±2.0	101.5±1.7*

*= P<0.0001 vs Basal

Table 4: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and during the Static hand grip exercise (peak) without HS

Parameter	HGE (n=20)	HGE +HS (n = 20)
SBP (mmHg)	146.8±2.7	139.1±2.0*
DBP (mmHg)	101.6±1.8	87.6±2.1*
MAP (mmHg)	116.3±2.1	104.8±1.9*
HR (beats/min	78.6±2.1	87.9±1.4*

*= p<0.0001 vs Basal

Table 5: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and during Static hand grip exercise (peak) in the presence of 15mg/kg *HS*

Parameter	Basal (N= 20)	CPT; PEAK (n=20)
SBP (mmHg)	131.8±2.2	147.1±2.4*
DBP (mmHg)	81.0±2.1	93.3±2.1*
MAP (mmHg)	97.9±2.0	111.1±2.2*
HR (beats/min	87.8±2.1	100.8±2.0*

*= p<0.0001 vs Basal

Also the peak values of these parameters obtained during the cold pressor test in the presence of HS were significantly (P<0.0001) higher than the basal values (Table 3). Figure 1 shows the changes between the peak value of each parameter and the basal value

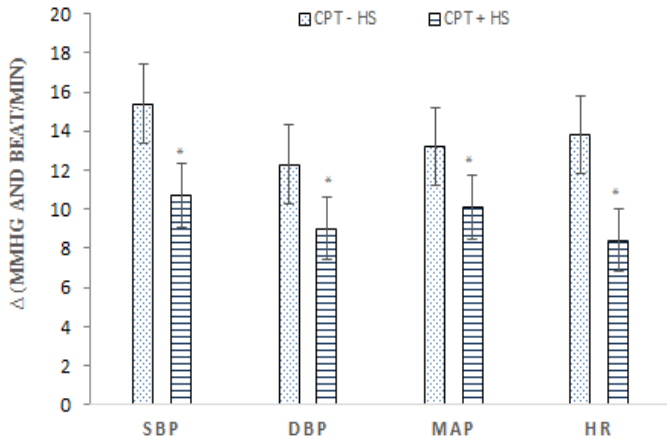

Figure 1: Change (Δ) between peak and basal values of each parameter during CPT with and without HS, *P<0.0001

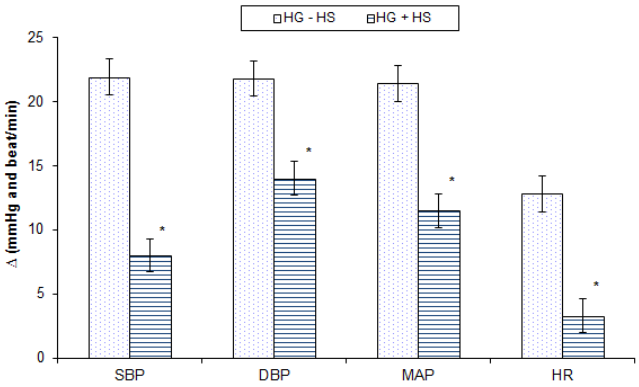

Figure 2: Change (Δ) between the peak and basal values of each parameter during HGE with and without HS, *p<0.0001

during the cold pressor test in the presence and absence of HS. The changes were all significantly (P<0.0001) lower in the presence of HS compared to its absence. Table 4 shows the blood pressure and heart rate values obtained during the static hand grip exercise in the absence of HS. All parameters rose significantly (P<0.0001) compared to the basal values. The parameters obtained during the static hand grip exercise in the presence of HS are presented in table 5. These parameters were all significantly (P<0.0001) higher than the basal values. Figure 2 shows the difference between the peak value of each parameter and the basal values during the static hand grip exercise in the presence and absence of HS. These changes were all significantly (P<0.0001) lower in the presence of HS compared to its absence.

DISCUSSION

The major finding of this study is that HS lowers blood pressure and heart rate through mechanisms associated with the sympathetic nervous system. These findings were obtained using protocols that are known to activate the sympathetic nervous system

such as the cold pressor test (Wood *et al.,* 1984; Halter *et al.,* 1984; Victor *et al.,* 1987; Seals, 1990) and the hand grip exercise (McCraw *et al.,* 1972; Chirinos *et al.,* 2009) The present study might be the first investigation to report that the BP lowering effect of HS may occur through the attenuation of sympathetic nervous system activities.

The blood pressure parameters (systolic, diastolic and mean arterial blood pressure) and heart rate were all raised significantly from the basal values during the cold pressor test. This suggests that the sympathetic nervous system was activated. Earlier studies have established that the pressor response to cold is sympathetically mediated (Wood *et al,* 1984; Victor *et al.,* 1987). Indeed, exposure to cold during CPT leads to stimulation of cold and pain receptors causing reflex vasoconstriction and increase in blood pressure and heart rate (Wood *et al.,* 1984). In the presence of HS the rise in these parameters were dampened. The difference between the peak values of these parameters and their basal values (change or Δ) were significantly lower in the presence of HS compared to its absence. This difference or change is a measure of the vascular reactivity to catecholamines released during the activation of the sympathetic nervous system by the cold stimulus (Wood *et al.,* 1984; Victor *et al.,* 1987). The significant dampening or reduction of these changes by HS suggests that its hypotensive effect may occur through the reduction of vascular reactivity during sympathetic nervous system activation.

During static hand grip exercise blood pressure parameters (systolic, diastolic and mean arterial blood pressure) and pulse rate were all raised significantly from the basal values. Again, this is indicative of sympathetic nervous system activation (Bruce *et al.,* 1972). These parameters were reduced in the presence of HS. Furthermore the difference between the peak values of these parameters and their basal values (change or Δ) were significantly lower in the presence of HS compared to its absence. This difference or change is due to significant increase in systemic vascular resistance during the activation of the sympathetic nervous system by the hand grip (Bruce *et al.,* 1972; Chirinos *et al.,* 2009). These cardiovascular adjustments to hand grip exercise are known to be regulated by the exercise pressor reflex which may be activated by the mechanically and metabolically sensitive receptors located in the nerve endings of group III and IV afferent sensory neurons respectively (Murphy *et al.,* 2011). Activation of this reflex leads to transmission of sensory impulses to cardiovascular control centres in the brain stem with subsequent activation of the exercise reflex resulting in an increase sympathetic nerve activity and withdrawal of parasympathetic nerve activity leading to exaggerated rises in sympathetic nerve activity, BP, HR and vascular resistance (Murphy *et al.,* 2011). Hence the attenuation of the BP and HR increases during the hand grip exercise in the presence of HS suggests that HS may be acting by dampening the sympathetic nerve activation or reducing the parasympathetic withdrawal that occur during handgrip exercise.

A comparison of the effect of HS on changes (Δ) in the measured parameters during CPT and HGE shows that the reduction in the change appears more marked in HGE compared to CPT (Figure 1 compared to Figure 2). This suggests that HS may be more effective in dampening sympathetic activation due to HGE than that due to CPT. This may be related to the fact that during HGE the arterioles are squeezed resulting in significant increase in systemic vascular resistance (Chirinos *et al.,* 2009). Since parasympathetic withdrawal is part of the mechanism for the pressor effect of HGE, it may also be speculated that the greater reduction in changes in the cardiovascular parameters may be due to a reduction in parasympathetic withdrawal by HS.

The results of this study are consistent with the earlier findings of Adegunloye *et al.,* (1996) which showed that HS lowers blood pressure via a vasodilatory effect. Such vasodilation may be achieved through acetylcholine-like and histamine-like mechanisms (Adegunloye *et al.,* 1996).

So far the only drug acting acutely to lower BP is sublingual Nifedipine (Furberg *et al.,* 1995). The present result suggests that, HS also lowers BP acutely. However, more studies are required to confirm this observation.

However, the present results are inconsistent with a finding by Adegunloye *et al.* (1996) which suggested that HS may not lower blood pressure by inhibiting the sympathetic nervous system. They used bilateral carotid occlusion (BCO) test to activate the sympathetic nervous system in rats. The blood pressure and heart rate responses to BCO in the presence of HS did not differ from the responses in its absence (Adegunloye *et al.*, 1996) making them to arrive at their conclusion. The differences in their results and the present one may be due to differences in techniques used to activate the sympathetic nervous system. Although BCO may be permissible in rats as a technique for activating the sympathetic nervous system, it is unethical in humans. The present study settled for CPT and HGE which are ethical in humans and have been used consistently by several authors in human subjects (Hines & Brown 1936; Bruce *et al.,* 1972; Wood et al., 1984 Chen *et al.,* 2008; Cui *et al.,* 2011). The difference between the result of Adegunloye *et al*. and the present one may also be due to species differences. Unpublished data from our laboratory suggest that responses to

BCO with and without HS are similar in rats thereby confirming the latter notion.

A dose of 15mg/kg body weight was used on account of its safety. A recent review by Hopkins *et al.*, 2013 reported that, HS extracts have a low degree of toxicity with LD50 between 2,000 to 5,000mg/kg/day.

The present study has some flaws. These include the lack of blinding of the BP and HR measurements as well as the use of a plier for the handgrip experiment instead of a dynamometer. The latter was not available and so we had to improvise. These flaws will be addressed in future.

In summary, the present study showed that the CPT and HGE induced increases in BP and HR were reduced in the presence of HS. It is concluded that the hypotensive effect of HS may be achieved through the inhibition of systemic vascular resistance mediated by the sympathetic nervous system.

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Effect of Mobile Phone Radiofrequency Electromagnetic Fields on Cardiovascular Parameters in Apparently Healthy Individuals

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Summary: Since cell phones emit radiofrequency electromagnetic fields (EMFs), this study tested the hypothesis that cell phones placed near the heart may interfere with the electrical rhythm of the heart or affect the blood pressure. Following informed consent, eighteen randomly selected apparently healthy male volunteers aged 21.44 ± 0.53 years had their blood pressure, pulse rates and ECG measured before and after acute exposure to a cell phone. The ECG parameters obtained were: heart rate (HR), QRS complex duration (QRS), PR interval (PR) and Corrected QT interval (QTc). Results are presented as mean ± SEM. Statistical analyses were done using two-tailed paired t test for blood pressure and pulse rate data and one way ANOVA with a post hoc Tukey test for the ECG data. $P<0.05$ was considered statistically significant. The blood pressure and pulse rates before and after exposure to the cell phone showed no significant difference. The ECG parameters (HR: beats/min, QRS:ms, PR:ms and QTc respectively) did not differ before (66.33 ± 2.50, 91.78 ± 1.36, 151.67 ± 5.39 and 395.44 ± 4.96), during (66.33 ± 2.40, 91.11 ± 1.61, 153.67 ± 5.06 and 394.33 ± 4.05) and after calls (67.22 ± 2.77, 91.11 ± 1.67, 157.44 ± 4.46 and 396.56 ± 4.93) compared to baseline (67.17 ± 2.19, 94.33 ± 1.57, 150.56 ± 4.93 and 399.56 ± 3.88). These results suggest that acute exposure to EMFs from cell phones placed near the heart may not interfere with the electrical activity of the heart or blood pressure in healthy individuals.

Keywords*:* Cell phone radio waves, Blood pressure, Electrocardiogramme.

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INTRODUCTION

There is a tremendous increase in the number of base stations and use of cell phones worldwide including developing African nations such as Nigeria. These base stations and cell phones emit radiofrequency electromagnetic fields (EMFs) and there are concerns about possible adverse effects on health. Recent studies in Nigeria (Ibitoye and Aweda, 2011) and Ghana (Deatanyah et al., 2012) suggest that EMFs emitted from base stations in the areas studied fall within the internationally recommended levels and may not, therefore, be harmful. However, there are still concerns that EMFs from mobile phones may interfere with body physiologic function. Evidence suggests, for instance, that EMFs may interfere with artificial cardiac pacemaker function (Censi et al., 2007). The brain may also get exposed to EMFs emitted from cell phones resulting in some minor alterations in function since cell phones are held to the ear close to the head during calls (van Rongen et al., 2009). A recent report suggested that short-term exposure to cell phone EMFs resulted in DNA single-strand breaks in hair root cells obtained from around the ear that was used for the phone call (Cam and Seyhan, 2012). The latter studies (van Rongen et al., 2009; Cam and Seyhan, 2012) suggest that proximity to EMFs from cell phones may not be as innocuous as generally believed.

In Nigeria, and probably other countries as well, men tend to keep their cell phones in the breast pocket close to the heart resulting in exposure to EMFs and probable untoward effects on the heart. However there are few studies that have examined this. Indeed to our knowledge only two studies (Barker et al., 2007; Barutcu et al., 2011) have examined the possible interference of EMFs from mobile phones on autonomic modulation of the heart. They assessed cardiac autonomic function by analysing heart rate variability (HRV) parameters (Barker et al., 2007; Barutcu et al., 2011). In the current study we tested the hypothesis that exposure to EMFs from cell phones placed close to the heart may interfere with the electrical rhythm of the heart and blood pressure. We did this by measuring the electrocardiogram (ECG) before, during and after exposure to a cell phone. In addition, we examined the effect of acute exposure to cell phone EMFs on blood pressure as a previous report suggested an

increase in blood pressure following exposure to EMFs from cell phones (Braune et al., 1998).

MATERIALS AND METHODS

This study involved eighteen randomly selected apparently healthy male volunteers. Their ages were recorded and their height (without shoes) and weight (with minimal clothing) were measured using an adult weight and height scale. Their body mass index (BMI) was calculated as weight (kg)/ height $(m)^2$. Following informed consent, they had 12-lead ECG performed on them in the Cardiology unit of the Usman Danfodio University Teaching Hospital (UDUTH) Sokoto, Nigeria. The recordings were done according to recommendations of the American Heart Association, American College of Cardiology and the Heart Rhythm Society (Kligfield et al., 2007). The Electrocardiograph (Dr. Lee, 310A, Korea) contains an in-built computer analysis programme (CAP) that delivers information about the heart rate and rhythm, frontal plane axis of the QRS, P and T-waves, durations of waves and intervals including the corrected QT interval (QTc). The CAP is also capable of detecting cardiac arrhythmias.

The subjects were not on any medication, and did not take alcohol or any caffeine containing drinks. They were also not involved in strenuous exercise at least 24 hours before the study. The study was done in a quiet dimly lit air conditioned room (temperature= 24^oC) between 2-5 pm daily to avoid circadian variations in blood pressure, pulse rate and ECG. The subjects rested for at least 30 minutes to acclimatize before recordings were made. The blood pressure was measured from the left arm with a mercury sphygmomanometer (Accoson, England) using standard procedures (Timmis, 2007) with the subject in the sitting position and the cuff at the level the heart. The systolic pressure was taken at the first appearance of the *Korotkoff* sound while the diastolic was taken at its disappearance corresponding to phases 1 and 5 respectively (Timmis, 2007). The pulse rate was measured using the radial pulse. Thereafter each subject was asked to lie on a comfortable couch for at least 15 minutes and the baseline ECG records were taken. Then a cell phone in the turned on mode was placed on the left chest wall lying astride the nipple and without contact with any of the electrodes, and recordings were made. Thereafter, recordings were made with the phone in the calling mode during which three calls were made from another phone three meters away. Finally, recordings were repeated with the phone in the turn on mode after the calls. The recordings for each phase (i.e. baseline, before calls, during calls and after calls) lasted five minutes. The blood pressure and pulse rate were measured again after the performance of the ECG procedure.

Statistical Analysis

Results are presented as mean ± SEM. The blood pressure and pulse rate data was analysed using two-tailed paired t test. The ECG data, as well as comparison of the pulse rates with the heart rates, were analysed using one way ANOVA with a post hoc Tukey test. $P<0.05$ was taken as statistically significant.

RESULTS

The age and anthropometric parameters of the subjects are presented in Table 1. The blood pressure and pulse rate before exposure to the cell phone did not differ significantly from values obtained after exposure (Table 2).

Comparisons of the ECG parameters obtained before, during and after calls showed no significant difference from baseline or from each other (Table 3). The pulse rates obtained (Table 2) were comparable to the heart rates obtained from the ECG (Table 3) at baseline, before, during and after exposure and they did not differ significantly from one another.

Table 1. The Age and Anthropometric Parameters of Subjects

Age (yr)	Ht (m)	Wt (Kg)	BMI (kg/m^2)
21.44± 0.53	1.68 ± 0.02	58.44 ± 1.43	20.66 ± 0.53

Table 2: The Blood Pressure and Pulse Rate of subjects before and after exposure to radiofrequency electromagnetic fields (EMFs) from a cell phone placed close to the heart.

Parameter	Before	After
Systolic Pressure (mmHg)	104 ± 2.71	103 ± 2.80
Diastolic Pressure (mmHg)	70 ± 1.74	68±1.52
Pulse Rate (beats/min)	69 ± 2.43	69±2.81

DISCUSSION

The results of this study show that the blood pressure, pulse rate and ECG of apparently healthy subjects did not change significantly following acute exposure to a cell phone. This suggests that acute exposure to EMFs from cell phones placed on the left chest wall within the surface anatomy of the heart may not

Table 3: The ECG of subjects at baseline, before, during and after calls were made to a cell phone placed close to the heart.

ECG Parameter	Baseline	Before Calls	During Calls	After Calls
Heart Rate (beats/min)	67.17 ± 2.19	66.33 ± 2.50	66.33 ± 2.40	67.22 ± 2.77
QRS Complex Duration (ms)	94.33 ± 1.57	91.78 ± 1.36	91.11 ± 1.61	91.11 ± 1.67
PR Interval (ms)	150.56 ± 4.93	151.67 ± 5.39	153.67 ± 5.06	157.44 ± 4.46
QTc	399.56 ± 3.88	395.44 ± 4.96	394.33 ± 4.05	396.56 ± 4.93

interfere with the normal electrical rhythm of the heart or affect the blood pressure and pulse rate regardless of the transmission mode.

Our findings on ECG are in agreement with those of Barker et al., (2007) and Barutcu et al., (2011) who studied the effect of EMFs emitted by mobile phones on autonomic modulation of the heart. They studied time and frequency domain heart rate variability (HRV) parameters before and after exposure to EMFs from a cell phone in different transmission modes. HRV was obtained by feeding the ECG data into a computer and using HRV software to obtain the time and frequency domain parameters (Barutcu et al., 2011). It is a very useful noninvasive technique for assessing autonomic cardiovascular function in the clinic and the time and frequency domain parameters obtained from it are recommended for short term recordings (Barutcu et al., 2011). Since such facilities were not available to us, we simply used resting 12-lead ECG parameters and compared them before, during and after exposure. Notwithstanding the differences in methods, our findings were similar.

The subjects studied were young apparently healthy male subjects with anthropometric parameters that fall within the normal values reported for Nigerians (Taura, 2011; Adediran, Adebayo and Akintunde, 2013) and had normal baseline ECG which was not influenced by acute exposure to cell phone EMFs. Our findings do not agree with the earlier report by Braune et al., (1998) which showed an increase in blood pressure following exposure to cell phone EMFs. A large double blind sham-controlled study (Barker, 2007) that sought to confirm the finding by Braune et al., (1998) reported, like the present study, no effect of EMFs on blood pressure.

The baseline ECG parameters of the subjects all fell within the normal range for black African males as reported by Araoye (2004). Values obtained during and after exposure also fell within the normal range and the sinus rhythm remained consistent confirming that acute exposure to EMFs from mobile phones placed close to the heart may not interfere with the electrical rhythm of the heart in healthy young men. The pulse rate was comparable to the heart rate before, during or after exposure regardless of the cell phone transmission mode suggesting that there was no atrial fibrillation. The period of exposure to the cell phone EMFs in this study (five minutes), fall short of the level of exposure occurring in men who keep their mobile phones habitually in the pocket. This is a limitation of this study and an earlier one by Barutcu et al., (2011). Hence it may be worthwhile to carry out a study with longer periods of exposure. However, a large double blind sham-controlled study by Barker et al., (2007) seemed to have addressed these issues. Their study involved measurement of blood pressure, heart rate variability and blood catecholamine in 120 healthy volunteers before and after six exposure sessions to different radio frequency signals from both GSM and TETRA handsets in different transmission modes (Barker et al., 2007). In spite of their comprehensive approach, with multiple exposure sessions for longer periods and with twenty four hour ambulatory recordings, no effect was found (Barker et al., 2007).

The lack of effect of acute exposure to EMFs emitted by GSM mobile phones on the ECG in this study suggests that acute exposure may be safe. Barutcu et al., (2011) have suggested that the safety of the mobile phones may be associated with their design. GSM mobile phones emit maximal power during the initiation of a call and then the radiofrequency waves fall to low levels sufficient to sustain the call (Barutcu et al., 2011). This ensures that exposures to EMFs are minimal during calls. However the effect of acute or chronic exposure remains to be determined in those with cardiovascular diseases.

Although the results of the present study suggest that acute exposure to EMFs from cell phones may be safe, a recent study has reported the presence of a subset of people that are hypersensitive to radiations from lap tops and or cell phones (Hänninen et al., 2013). The symptoms reported included cooling of the hands when holding a cell phone or typing on a lap top and a transient increase in blood pressure following a short exposure to a cell phone in a volunteer with a cardiac pacemaker. The cooling of the hands and the rise in blood pressure were attributed to the effects of EMFs on the sympathetic nervous system (Hänninen et al., 2013). They concluded that unnecessary exposure to radiations from computers and cell phones should be avoided.

In conclusion, our data suggest that acute exposure to mobile telephony placed within the surface anatomical location of the heart may not alter the blood pressure, pulse rate and the electrical rhythm of the heart in healthy individuals.

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The Effect of Intranasal Desmopressin Spray on Basal and Total Tear Secretions in Healthy Subjects

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Summary: Many hormones have been implicated in dry eye syndrome. This study was carried out to investigate the effects of antidiuretic hormone (ADH) on tear secretion. Fifty (50) healthy male and female volunteers between the ages of 21 and 70 years were studied. They were given exogenous ADH in the form of intranasal desmopressin spray (DDAVP). Total and basal tear secretions were assessed using the Schirmer strip at baseline and at 30 minutes intervals for a period of 180 minutes after the nasal administration of 10 µg desmopressin spray into each nostril. Blood samples were taken before and 60 minutes after desmopressin administration for the determination of plasma osmolality and plasma ADH concentrations. Results showed a significant reduction ($p<0.001$) in the means of total and basal tear secretions from baselines of 20.04 ± 1.19 and 14.64 ± 1.00 mm/5mins to 12.80 ± 0.75 and 9.68 ± 0.72 mm/5mins peak reductions respectively. The peak reduction was observed at 90 minutes assessment time after desmopressin administration. The difference in mean total tear secretions between males and females were statistically significant ($p < 0.05$). The difference in mean plasma osmolality before and 60 minutes after desmopressin administration was not statistically significant. However, there was a significant increase ($p<0.05$) in mean ADH concentration after DDAVP administration. It is concluded from this study that exogenous ADH reduces tear secretion in man with associated increase in ADH concentration but no change in plasma osmolality.

Keywords*:* Tears, Desmopressin, Osmolality, Antidiuretic hormone, Schirmer strip, Lacrimal gland

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INTRODUCTION

Antidiuretic hormone (ADH) also known as arginine vasopressin in humans is a peptide consisting of nine amino acids (nonapeptide) produced in the supraoptic and paraventricular nuclei of the hypothalamus, transported to and stored in the posterior pituitary. ADH is responsible for increasing water absorption in the collecting ducts of the kidney nephron which confers its major role in the regulation of water balance (Nielsen *et al*, 1993). ADH is also known as vasopressin because of the vasopressor effect at pharmacological doses.

Desmopressin is a synthetic analogue of the natural pituitary hormone-ADH or better still; it is a synthetic replacement for vasopressin. Compared to vasopressin, desmopressin's first amino acid has been deaminated, and the arginine at the eighth position is in the *dextro* rather than the *levo* form. It is also known as 1- desamino (8 - D- arginine) vasopressin (DDAVP). Desmopressin works by binding to the ADH receptors in the kidneys, which mimics the effect of natural ADH and this reduces the production of urine *(*den Ouden *and* Meinders, 2005). Desmopressin is degraded more slowly than vasopressin, and requires less frequent administration. In addition, it has little effect on blood pressure, while vasopressin may cause arterial hypertension.

The preocular or precorneal tear film is a thin film of tears covering the anterior surface of the cornea. The preocular tears form a film between the inside of the lids and the cornea when the eyes are closed and remain as a film over the cornea for a limited time after the lids are opened [usually greater than 15 seconds] (Whikehart, 2003). The tear film performs several important functions which can be divided broadly into optical, metabolic support, protective and lubricative functions. Complete tear film is essential for the health and function of the eye (Walcott, 1998). Normal tear film dynamics require adequate production of tears, retention on the ocular surface, and balanced elimination. Disruption of any of these components can lead to the condition of dry eye (Tomlinson and Khanal 2005). Dry eye syndrome also known as "keratoconjunctivitis sicca" occurs when there is reduced tear production, unstable tears or excessive tear evaporation and it is the most common disorder of the tear film. Keratoconjunctivitis sicca (KCS) refers to a dry eye primarily resulting from aqueous tear deficiency (Lemp, 1980). Dry eye syndrome is believed to be one of the most common ocular problems in the United States of America (Schaumberg *et al*., 2003).

Many hormones, for example, sex hormones have been implicated in dry eye syndrome, and the presence of antidiuretic hormone in the lacrimal gland of the rat has been documented immunohisto-chemically (Djeridane, 1994). There are conflicting results in the few available data on the effects of ADH on tear dynamics. Turkcuoglu, (2006) in his preliminary study on the effects of desmopressin on aqueous secretion of the lacrimal gland showed that desmopressin decreases the lacrimal glands aqueous secretion. Kurasawa *et al.* (2005) however reported that exogenous ADH increases tear fluid secretion in rats via ADH V1a receptors.

The aim and objectives of this research were to investigate the effect of antidiuretic hormone (ADH) on tear secretion, determine if the effect of ADH on tear secretion is dependent on gender and also determine the plasma concentrations of ADH following intranasal desmopressin administration.

MATERIALS AND METHODS

This study was carried out in the Department of Optometry, University of Benin, Benin City, Nigeria. The study included Fifty (50) apparently healthy volunteers between the ages of 21 and 70 years, comprising 27 males and 23 females. These included staff and students of the University of Benin, Benin City, and staff of Specialist Hospital Benin City, Nigeria.

The study procedure was well explained to each participant and informed consent was obtained from all of them. The study was approved by the ethics committee of the Specialist Hospital, Benin City and was performed in accordance with the guidelines of the Declaration of Helsinki.

Inclusion criteria

The subjects included in the study were males and females within the age range of 21 and 70 years, healthy subjects with no history of systemic diseases and ocular diseases, subjects who wore contact lenses and those who were using any form of topical or systemic medication within 30 days were excluded from the study.

General procedures

A brief case history was taken to provide information on subjects' ocular health and general health. Visual acuity test was carried out using the standard Snellen's chart. The slit lamp biomicroscope (Zeiss, USA) was used to examine the external segment of the eye to rule out ocular surface and anterior segment abnormalities. The monocular direct Ophthalmoscope (Keeler, UK) was used to examine the internal structures of the eye to rule out diseases of the posterior segment. Blood pressure was measured with U-MEC mercurial sphygmomanometer and Sprague stethoscope (Model No 112) to rule out hypertensive patients from the research study.

Subjects who met the inclusion criteria were recruited for the study.

Data collection

With each subject comfortably seated in the examination room, the baseline measurements of total and basal tear secretions were taken as described below. Blood samples of each subject were then taken for determination of baseline plasma osmolality and plasma concentration of ADH. Blood was drawn from the ante-cubital vein of each subject into chilled EDTA specimen bottles for determination of plasma ADH concentration, into lithium heparin bottles for determination of plasma sodium and potassium concentrations and into sodium oxalate bottles for the determination of plasma urea and glucose concentrations.

After 10 minutes rest, a spray dose of 10 µg was administered into each nostril (as described below) giving a total of 20 µg per subject. Thirty (30) minutes after administration of DDAVP, blood pressure measurements, total tear secretion and basal tear secretion were taken and were repeated at 30 minutes interval until 180 minutes.

One hour post administration of desmopressin nasal spray, which is the time for the most effective serum concentration of desmopressin (Den Ouden and Meinders, 2005), blood samples were taken again for determination of plasma osmolality and plasma concentration of ADH.

Samples were sent to the laboratory within 30 minutes of collection.

Measurement of tear secretions

Total tear secretion

Total tear secretion was measured by Schirmer test (without anaesthetic) and it was done monocularly (right eye only) (Turckuoglu, 2006). The subject was comfortably seated and was instructed to look up. The lower eyelid was gently pulled down, the bent hooked end of the Schirmer strip was then placed at the junction of the middle and outer two third of the lower eyelid taking care not to touch the cornea or lashes. The subject was then instructed to close the eye. The time of insertion was noted and after 5 minutes the strip was removed and the wetted portion of the strip from the notch towards the flat end was measured in mm and recorded. Length of wetting of less than 10mm in five minutes was considered low tear secretion.

Basal tear secretion

After 10 minutes of rest, one drop of local anaesthetic (0.5% tetracaine) was instilled into the lower conjunctival fornix of the left eye (OS) and the above procedure repeated. This value gave the basal tear

secretion rate. Length of wetting of less than 10mm in five minutes was considered low tear secretion.

Intranasal application of Desmopressin (DDAVP) Nasal Spray 0.1mg/ml

Desmopressin which is a synthetic analogue of ADH was used in this study.

Desmopressin (DDAVP) Nasal Spray (Ferring, GmbH, Germany) contains desmopressin acetate at a concentration of 0.1mg/ml. Each ml contains 0.1mg desmopressin acetate equivalent to 0.089 mg DDAVP (desmopressin). The vial is equipped with a snap-on, tamper-proof precompression pump which gives a spray dose of 10 µg desmopressin acetate.

The pump of the spray was primed by pressing downwards until an even spray was obtained. This was to ensure that the spray delivered 10 µg each time it was pressed. The protective cap was then removed and it was ensured that the end of the tube inside the bottle was totally submerged in the liquid.

The head of the subject was tipped back slightly while the applicator was inserted into the nostril. A spray dose of 10 µg was administered into each nostril giving a total of 20 µg per subject.

Hormonal Assay

Plasma concentrations of ADH were assessed using Assay Designs' arginine Vasopressin Enzyme Immunoassay (EIA) kit Ann Arbor, MI. Catalog No. 900-017, Lot No. 08040908.

Plasma osmolality

Plasma osmolality was estimated by the summation of urea, glucose and twice sodium and potassium as shown below.

Calculated osmolality (mOsmol/kg) = 2 Na + 2 K + Glucose + Urea (all in mmol/L) (Purssell *et al.*, 2001).

Flame photometry was used for the estimation of sodium and potassium in plasma, urease method was used for the estimation of urea while glucose oxidase method was used for the estimation of glucose in the number of samples.

Statistical analyses

Results are presented as means and standard error of means. All data obtained in this study were analyzed using SPSS 17.0, SPSS Inc., Chicago, IL, USA. and STATGRAPHICS Centurion XVI, Dayton, OH, USA. Pre desmopressin and post desmopressin results were analyzed using the paired *t* - test to determine the effects of desmopressin on plasma osmolality and plasma ADH concentration using SPSS 17.0. Unpaired *t* – test was used to determine significant differences in means of tear secretions in males and females (SPSS 17.0). Analysis of variance (ANOVA) was used to determine the effects of desmopressin on tear secretion across different assessment times, and Fisher's least significant difference (LSD) was used to identify the highest significant mean differences. (STATGRAPHICS 5.1). Significance was declared when probabilities values were, $p < 0.05$.

RESULTS

The mean age of subjects in the total population was 40.75 ± 2.61, (males =42.11 ± 2.52, females =39.39 ± 2.71). As shown in fig.1, there was a gradual reduction in total tear secretion from baseline value of 20.04 ± 1.19 mm/5mins which showed a significant reduction at 90 minutes with a mean of 12.80 ± 0.75 mm/5mins (p<0.001). Thereafter, there was a gradual increase with a mean of 19.30 ± 1.11 mm/5mins at 180 minutes. Similarly, there was a significant difference in mean basal tear secretion across different assessment time, (P< 0.001) with peak reduction at 90 minutes with a mean of 9.68 ± 0.72 mm/5mins from baseline of 14.64 ± 1.00 mm/5minutes. There was also a gradual increase of up to 13.82 ± 0.95 mm/5mins at 180 minutes (Fig.2)

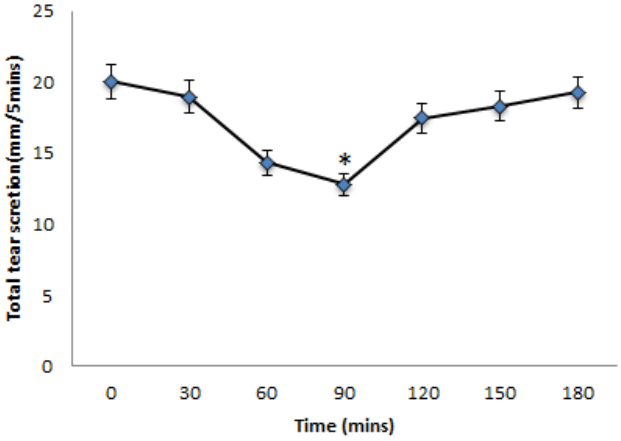

Fig.1: Mean total tear secretion at baseline and at different assessment times after desmopressin administration in the total population. * p<0.001

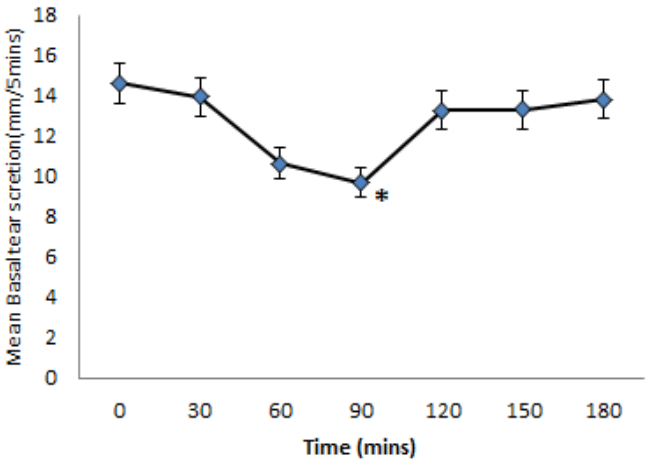

Fig.2: Mean basal tear secretion at baseline and at different assessment times after desmopressin administration in the total population. * p<0.001

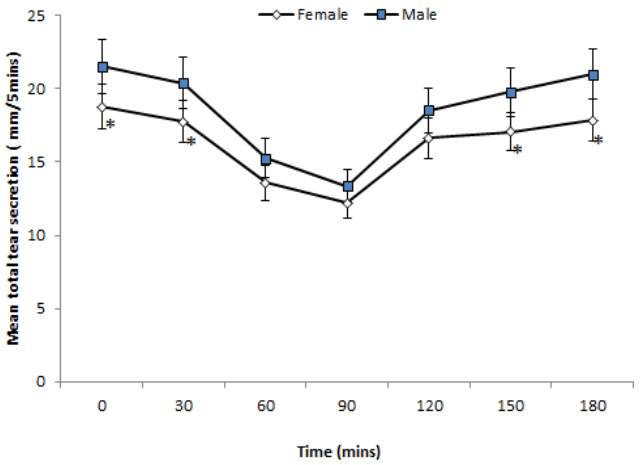

Fig.3: Mean total tear secretion at baseline at different assessment times after desmopressin administration in males and females

Table 1: Mean Basal tear secretion at baseline and at different assessment times after desmopressin administration in males and females

Time (mins)	Mean Basal Tear Secretion (mm/5minutes)	
	Males (n=27)	Females (n=23)
0	15.60±1.47	13.81±1.37
30	15.13±1.50	12.88±1.30
60	11.47±1.15	9.88±1.07
90	10.30±0.99	9.14±1.04
120	14.34±1.44	12.37±1.27
150	14.17±1.44	12.59±1.28
180	14.73±1.43	13.03±1.29

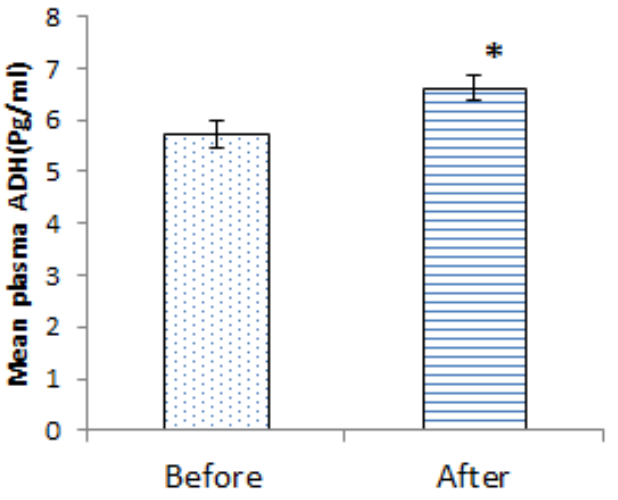

Fig. 4: Mean plasma ADH before and after desmopressin administration in total population. *p < 0.001

The total tear secretions in female subjects were significantly decreased (p <0.05) at baseline, 30 minutes, 150 minutes and 180 minutes when compared with the male subjects (fig 3). However there was no difference in the basal tear secretions of the two genders (table 1).

As shown in fig 4, mean plasma ADH concentration increased significantly from baseline value of 5.72 ± 0.25 pg/ml to 6.62 ± 0.25 pg/ml after

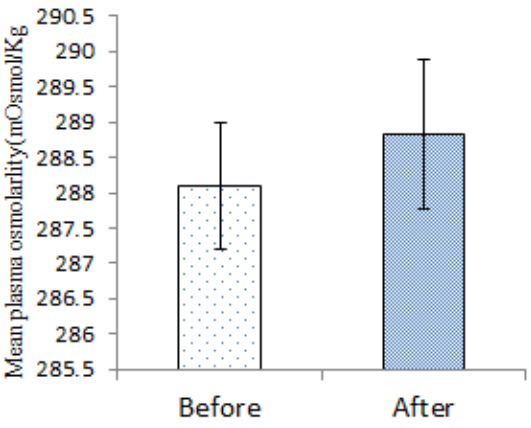

Fig.5: Mean plasma osmolality before and after desmopressin administration in total population

DDAVP (p < 0.001) and there was no significant difference in mean plasma osmolality before (288.09±0.89 mOsmol/Kg) and after (288.83±1.06 mOsmol/Kg) DDAVP administration (fig 5).

DISCUSSION

Complete tear film is essential for the normal health and function of the eye (Walcott, 1998). Normal tear film dynamics require adequate production of tears, retention on the ocular surface, and balanced elimination. Disruption of any of these components can lead to the condition of dry eye (Tomlinson and Khanal 2005).

In the present study, desmopressin intranasal spray significantly reduced the total and basal tear secretions with a peak reduction at 90 minutes after administration as shown in Figures 1 and 2. This finding is consistent with the findings of Turkcuoglu, (2006) who reported a decrease in lacrimal aqueous secretion after the administration of intranasal desmopressin in humans. They however suggested further studies to be able to identify the mechanism of action responsible for this decrease. Kurasawa, *et al*., (2005) however reported an increase in tear fluid secretion following an intravenous administration of ADH in rats. They demonstrated that a selective V_{1a} receptor antagonist reduced tear secretion in the same animal models. They therefore implicated the V_{1a} receptors for this increase in tear secretion.

It has been reported that peptide agents and humoral factors all play a role in modulating the processes of tear secretion from the lacrimal glands (Dartt, 1989). Studies by Affinitio, *et al*., (2003) have demonstrated that sex hormones play an important role in the secretory functions of the main lacrimal gland. A variety of medications for various conditions are known to be inhibitory to tear production. These drugs comprise of ganglion blockers, hypnotics and sedatives, antihistamines oral contraceptives, etc. (Van Haeringen, 1997; Crandall and Leopold, 1979). The lacrimal gland is tubulo-

acinar with large number of secretory acini. There are also myoepithelial cells closely associated with the secretory cells which squeeze the secretory products down the tubule. In normal tear secretion of the lacrimal gland, water a major secretory product of the lacrimal gland moves from the interstitial spaces into the lumen where it is mixed with other secretary products. The movement of water is driven by osmosis which depends on the movement of ions from the acinar cells into the lumen of the gland (Crandall and Leopold, 1979). The acinar cell surface membrane is differentiated into apical domain which contain water channels, aquaporin 5, which facilitate the movement of water across the epithelium, and the basolateral membranes which contain large numbers of $Na^+ – K^+$ pump which maintain the usual gradients that are seen in all cells. The movement of ions into the lumen will osmotically drive the movement of water into the aquaporin water channels into the lumen to maintain the osmotic balance (Walcott, 1998). Tearing is a form of water loss. ADH conserves water by preventing water loss from the body by reabsorption of water through the Kidneys.

The mechanism for the reduction of tear secretion by ADH is not well understood (Turkcuoglu, 2006). However, since desmopressin is a V_2 agonist, with its main function being antidiuresis, we hypothesized that it brings about its action by increasing the permeability of the apical and basolateral membranes of the acinar cells thereby pulling water from the lumen into the interstitium. This therefore brings about the reduction in tear secretion. This result therefore suggests that ADH does not only reduce water loss through the kidney, it also does it through reducing water loss in the form of tear secretion.

The peak reduction in tear secretion was observed at 90 minutes after which there was a gradual rise in tear secretion which almost reversed to the baseline values. This observation is consistent with the reports that DDAVP intranasal spray reaches a peak plasma concentration at about 1 – 1.5 hours (Harris, (1989). This observation suggests that the effect of desmopressin may be within 180 minutes in the ocular tissues. This is also consistent with the reports of Bitchet *et al*, (1988) who found out that the effect of DDAVP on mean arterial blood pressure reversed back to normal over the following 60 – 90 minutes. These observations show that desmopressin reduced the secretions of both the main lacrimal gland and accessory lacrimal glands as the main lacrimal gland is responsible for reflex tears while the accessory glands of Krause and Wolfring are responsible for basal tears.

The observation of reduction in tear secretion by desmospressin suggests that it predisposes to dry eye Syndrome, Keratoconjunctivits Sicca (KCS). This condition occurs when there is reduced tear production, unstable tears or excessive tear evaporation. Keratoconjunctivitis sicca is a dry eye primarily resulting from aqueous tear deficiency (Walcott, 1998; Lemp, 1995). Aqueous-deficient dry eye is due to a lack of tear secretion from the lacrimal glands.

The significantly lowered total tears secretion observed in this study is consistent with the reports of other researchers who reported decreased tear secretions in women compared to their male counterparts and the higher prevalence of dry eye syndrome amongst women (Schaumberg *et al*, 2001). The female gender has been identified as a risk factor for dry-eye development (Caffary *et al*, 1996). These reporters related this high prevalence to the findings that 90% of the individuals with primary or secondary Sjogren's syndrome are women, and that these auto immune disorders are among the most frequent causes of aqueous-deficient dry eye. It has been documented that the onset of dry eye is very common during menopause and may result from the loss of hormonal support (Gupta, 2006). In humans, tear production is correlated with serum prolactin and sex steroids hormone levels prior to and during menopause (Mathers *et al*, 1998). These findings are consistent with the findings of Idu and Oghre (2010) who reported a decrease in tear secretion of females compared to males.

The results of this research showed a significant increase in plasma ADH concentration from baseline levels after desmopressin administration. DDAVP intranasal spray is absorbed from nasal mucosa into plasma. The time to peak effect is 1 – 1.5 hrs (Harris, 1989). Vilhardt and Lundin, (1986) in a study comparing the biological effect and plasma concentration of DDAVP after intranasal and peroral administration to humans, reported that 11.3% of the dose of DDAVP through intranasal administration appeared in the blood thereby significantly increasing the plasma ADH level from baseline values. Chiozza *et al*, (1998) in a study of evaluation of ADH before and after treatment with desmopressin in a group of enuretic children reported a significant increase in plasma ADH levels at the end of treatment. These findings suggest a good bioavailability of desmopressin in plasma following intranasal administration.

This study showed no significant change in plasma osmolality after desmopressin administration at the concentration used for this study. This observation is consistent with the findings of Wallace *et al*, (1988). Wong *et al*, (2003) also reported that DDAVP tablet had no effect on plasma osmolality and plasma electrolyte levels (found no significant difference in osmolality, sodium or potassium levels between pre and post infusion of DDAV in human subjects).

In conclusion, this study showed a significant reduction in both total and basal tear secretions. This

reduction was however transient as the tear values returned to almost baseline values at 180 minutes. This demonstrates that antidiuretic hormone (ADH) reduces tear secretion hence, the use of medications containing ADH agonists may predispose users to dry eye syndrome.

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Antioxidant Effects of Methanol Extract of *Allium cepa linn* on Cyanide-induced Renal Toxicity in Male Wistar Rats

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Summary: The protective effects of onion was assessed in the Kidney of rats following sub-acute exposure to cyanide. These effects were compared to those of sodium thiosulphate ($Na_2S_2O_3$), a classical antidote of cyanide toxicity. The rats were divided into 6 groups of 6 animals each. Group 1 was administered distilled water, Group 2,3,4, 5, and 6 were administered 600 mg onion/kg bwt/day, 7 mg KCN/kg bwt/day, 300 mg onion/kg bwt/day+ 7 mg KCN/kg bwt/day, 600 mg onion/kg bwt/day+ 7mgKCN/kg bwt/day, 600mg $Na_2S_2O_3$/kg bwt/day+ 7mgKCN/kg bwt/day respectively for 2 weeks. Group 3, 4 and 5 were pre-administered with 300mg onion/kg bwt/day, 600mg onion/kg bwt/day and 600mg $Na_2S_2O_3$ /kg bwt/day respectively for 2 weeks. Serum and urine creatinineand urea level were assessed as a measure of kidney function. Oxidative stress and antioxidant parameters were estimated in Kidney. Serum creatinine and urea levels were significantly higher in the cyanide treated rats compared with control. This was accompanied by significant reduction in the urine level of creatinine and urea. Co-administration with onion extract and $Na_2S_2O_3$ reverse the situation in both the serum and urine. The level of malondialdehyde (MDA) in rats treated with cyanide (3.846±0.20µg/g) was significantly increased in the kidney relative to control (0.691±0.15µg/g). This was accompanied with a decreased in antioxidant enzymes Superoxide Dismutase (SOD) (2.0±0.09U/mg), Catalase (CAT) (0.014±0.001katf),Glutathione-S-Tranferase (GST) (0.015±0.009nMol/mg) and non-enzymatic antioxidant Reduced Glutathione (GSH) (4.006±0.09µg/ml) compared with control (4.8±0.13 U/mg, 0.047±0.001 katf, 0.022±0.0013 nMol/mg, 6.802±0.2 µg/ml respectively). Co-administration with onion extract and $Na_2S_2O_3$ significantly increased these antioxidant enzymes and significantly decreased the concentration of malondialdehyde in the kidney. The results indicate that onion extract reduced lipid peroxidation in the kidney and increased antioxidant status of animals exposed to cyanide in a dose dependent manner

Keywords*:* Onion, Sodium thiosulphate, Lipid peroxidation, Antioxidant, Cyanide.

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INTRODUCTION

Cyanide is a serious environmental pollutant that is extremely toxic to several forms of life because of its inhibitory activity on a variety of key enzymes (Cipollone et al., 2007). One major enzyme inhibited by cyanide is the cytochrome c oxidase enzyme, in which it prevents cellular oxygen utilization and ATP production (Way, 1984).

Cyanide poisoning may result from exposure to hydrocyanic acid and cyanide salts (Benaissa et al, 1995). Cyanide is found in a number of plants such as cassava roots, in the form of cyanogenic glycosides (Vetter, 2000). In cases of fatal oral poisoning cyanide has been detected in the brain, blood, liver and also kidney (Ansell and Lewis, 1970).

The mechanisms by which cyanide exert its effects have not all been fully elucidated. Although in the inhibition of the mitochondria respiratory chain, cyanide produces the superoxide anion.Cyanide has been shown to induce oxidative stress (lipid peroxidation) in the brain of acutely treated mice (Mills et.al., 1996). Some of its biochemical effects could therefore be mediated through the generation of reactive oxygen species on some target organs such as the kidney (Okafor et. al., 2006). Exposure to high levels of ROS leads to depletion in antioxidant levels in animals. One of these antioxidants in the body is reduced glutathione, a reducing agent in biological cells that provides primary antioxidant defence against reactive intermediates of metabolism, drugs or carcinogens (Meister and Anderson, 1983). Cyanide is also a potent inhibitor of the enzymatic antioxidants catalase, superoxide dismutase, and glutathione peroxidase (Kanthasamy et al, 1997).These mechanisms may function in concert to produce the oxidative stress and damage seen after cyanide exposure.

Allium cepa linn (onion), is used as foodstuff, condiments, flavouring, and in folk medicine. Onions have been extensively studied for their therapeutic uses as antibiotic, antidiabetic, antiatherogenic, anticancer etc (Augusti, 1996). Onion oil has been reported to effectively decrease the lipid levels in experimental animals (Bobbi et al., 1984). It has been found that administration of onion products to diabetic rats significantly reduced hyperglycaemia (Kumud et al., 1990). Biological action of *Allium*

products is ascribed to organosulfur compounds, which have also been shown to possess antioxidant and free radical scavenging activities. Onions have previously been shown to protect testis against cadmium induced oxidative stress in rats (Ola-Mudathir et al., 2008). It is therefore hypothesized that onion extract could exert a protective effect against cyanide induced oxidative stress in the kidney.

Thus, this study investigates the protective effects of onion against cyanide induced oxidative stress in the kidney of wistar rats.

MATERIALS AND METHODS

Plants collection and preparation of extracts

Fresh bulbs of red onions were collected from Bodija market, Ibadan Nigeria and authenticated in the Department of Botany, University of Ibadan.

Extraction of plant material

The onions were washed with distilled water and allowed to air dry for one hour. The outer covering of the onion were manually peeled off. The onion bulbs being separated were washed and extracted in the following ways: Exactly 786g of fresh onion bulbs were blended on ice and soaked in 1000 ml of absolute methanol for 72hr in a clean, glass container and was filtered using a sterile muslin cloth after which the extract obtained was dried using the rotatory evaporator and stored in a refrigerator until required.

Experimental procedure

Thirty six male albino rats obtained from the animal colony of the Central Animal House of University of Ibadan were used. All animals were kept at room temperatures and had free access to drinking water and their pellets. The animals were acclimatized for two weeks to their environment before experimentation. All animal experiments were conducted in accordance with the International Ethical Norms on Animal Care and Use as contained in NIH publication/85-23, revised in 1985.

The rats were grouped into six groups (1, 2, 3, 4, 5 and 6) of six rats each. Group 1 served as control and received 0.2ml /day distilled water. Group 2 animals were treated orally with methanolic extract of Allium cepa *(*600mg/kg bwt/day*).* Group 3 was treated orally with potassium cyanide (7mg/kg bwt). Group 4 animals were treated orally with methanolic extract of Allium cepa (300 mg/kg bwt/day) and potassium cyanide (KCN) (7mg/kg bwt/day). Group 5 was treated orally with aqueous extract of *Allium cepa* 600 mg/kg bwt/day + 7mg/kgbw/day KCN. Group 6 was treated orally with thiosulphate 600mg/kg bwt/day + 7mg/kg bw/day KCN. Group 4, 5 and 6 were pre-administered with 300mg onion/kg bwt/day, 600mg onion/kg bwt/day and 600mg $Na_2S_2O_3$ /kg bwt/day respectively for 2 weeks before co-administration of onion and potassium cyanide. The animals were observed daily and weighed at intervals of 2 days for two weeks. At the end of the experiments, the animals were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected into plane tubes and centrifuged at 1500g for 10minutes to obtain serum for biochemical analysis. The kidneys were removed into ice-cold container for evaluation of oxidative stress and antioxidant enzymes. Tissue sample for histology was fixed in 10% saline formalin.

Biochemical analysis

Level of Lipid peroxidation was evaluated by method of Buege and Aust (1978). Catalase activity was evaluated by method of Ashru and Sinha (1971). SOD activity was evaluated by method of Misra and Fridovich (1972). GST activity was evaluated by the method of Habig et al (1974). Protein estimation was done by method of Lowrey (1951). GSH content was evaluated by the method of Beutler et al (1963). Serum and urine creatinine and urea were evaluated using Randox® creatine and urea kits, respectively.

Histology

Histology of Kidney was done using H and E staining techniques.

Statistical Analysis

Data were presented as Mean ± SEM Student t test and ANOVA were used for comparison of results. $P< 0.05$ was considered significant.

RESULTS

Relative tissue weight

Relative kidney weight was significantly higher in the cyanide treated group ($P<0.05$) when compared to the control and co-administration of onion and Sodium thiosulphate did not produce any significant change (Table1).

Serum and urine analyte

Serum urea and creatinine level was significantly increased in the cyanide treated group ($p<0.001$) compared to control and this was reduced significantly by Co-administration with onion and sodium thiosulphate ($p<0.001$), while Urine creatinine level was significantly lowered in the cyanide treated group ($p<0.001$) compared to control. Co-administration with onion and sodium thiosulphate, however significantly increase it ($p<0.001$) (Table 3). Urine urea level was significantly lower in the cyanide treated group ($p<0.001$) compared to control and it was raised significantly by Co-administration of onion extract and sodium thiosulphate ($p<0.001$) (Table 1).

Oxidative stress Marker in the Kidney

Kidney malondialdehyde were significantly higher in the cyanide treated group (P<0.001) compared to the control and were reduced significantly by co-administration with onion extracts and sodium thiosulphate (p<0.001). Kidney SOD activities was significantly lowered in the cyanide only group compared to control (p<0.001) and was significantly increased by co-administration with the onion extracts (p<0.001). Co-administration of sodium thiosulphate however does not have any significant affect (Table 2).

Catalase activities in the kidney was significantly lowered in the cyanide only group compared to control (p<0.001) and was significantly increased by co-administration with the onion extracts and Sodium thiosuphate (p<0.001) (Table 2). Kidney GST activities was significantly lower in the cyanide only group compared to control (p<0.01) and co-administration with 600mg/kg bwt onion extract and sodium thiosulphate significantly increase it (p<0.01) (Table 2). Kidney GSH activities was significantly reduced in the cyanide only group with respect to the control (p<0.01) and was increased significantly by co-administration with 600mg/kg bwt onion extract and sodium thiosulphate (P<0.01), co-administration with 300mg/kg bwt produced no significantly affect (Table 2).

Histopathology of the Kidney

As shown in Figure 1A, B and E, kidneys of the control, onion extract only and 600mg/kg bwt onion co-administered with cyanide treated animals had intact cyto-architecture, glomeruli and collecting ducts. Fig 1C shows acute tubular necrosis for cyanide only group. Fig 1D and F also shows normal glomeruli with very mild focal tubular necrosis for 300mg/kg bwt onion extract and sodium thiosulphate co-administered with cyanide group respectively.

DISCUSSION

The present study shows that sub-acute cyanide toxicity increased the serum urea and serum creatinine level. One major function of the kidney is clearance of metabolites from the blood (Guyton and Hall, 2006). Agents with nephro-toxicity effects may raise serum creatinine and urea level. In addition cyanide decreased the urine creatinine and Urea level, suggesting that glomerular filtration rate might have been impaired. However, these effects were ameliorated by co-administration of onion extract or $Na_2S_2O_3$.

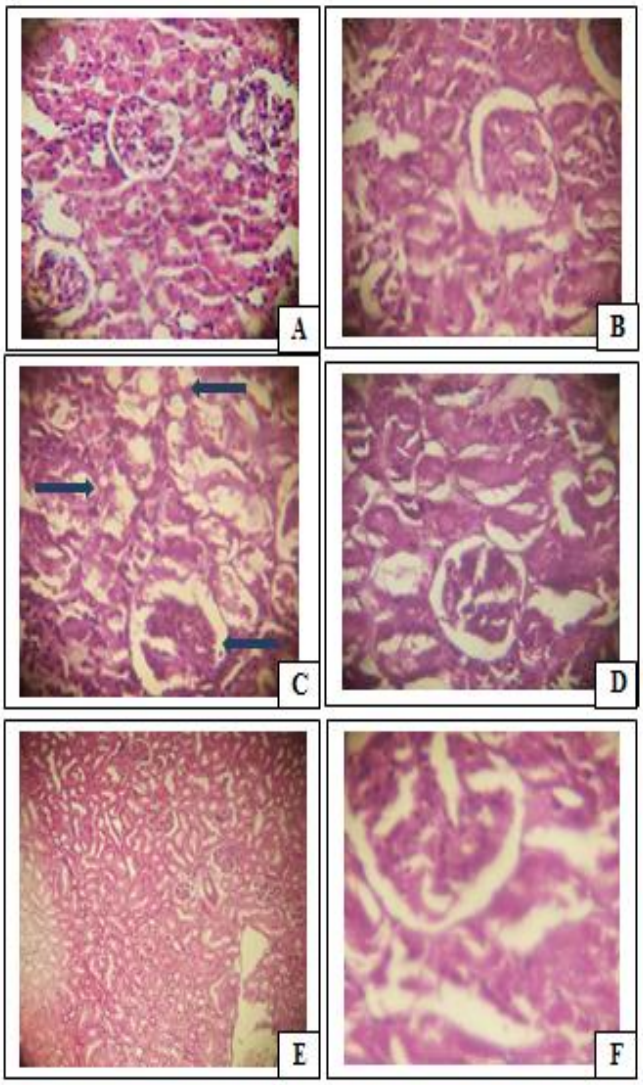

Fig 1. Photomicrograph showing transverse section of the kidney in (A) control animals (B) Alium cepa only (C) CN only treated with acute tubular necrosis (D) 300mgAlium cepa +KCN with mild focal tubular necrosis (E) 600mg Aliumcepa+ KCN (F) Sodium Thiosulphate+ KCN with mild focal tubular necrosis.

Table 1: Effects of methanol extract of *A. Cepa* on relative organ weight and serum and urine creatinine and urea.

Groups	Relative Organ wt	$S_{creatinine}$(µmol/l)	$U_{creatinine}$(mmol/l)	S_{urea}(mmol/l)	U_{urea}(mmol/l)
1	6.07±0.14	73.19±3.37	1.515±0.004	4.618±0.40	9.476±0.30
2	6.02±0.21	74.21±2.14	2.31±0.4	5.120±0.30	8.760±0.31
3	6.65±0.19 *	134.53±2.73 *#	0.529±0.004 *#	7.650±0.35 *#	3.204±0.37 *#
4	6.14±0.31	92.63±2.07	3.256± 0.675	7.268±0.27	5.84±0.24
5	6.22±0.31	96.32±2.04	2.93±0.41	7.290±0.23	4.970±0.31
6	6.19±0.24	81.32±0.12	4.21±0.432	7.213±0.32	5.453±0.341

1= Control, 2=Onion Only, 3=Cyanide Only, 4=Cyanide+300mg onion extract, 5=Cyanide+600mgonion extract, 6=Cyanide+Sodium thiosulphate. S= Serum, U= Urine, *p< 0.05 (compared to control), #p<0.05(compared to Cyanide+600mg onion extract).

Table 2: Effects of methanol extract of *A. Cepa* on Kidney MDA, GSH, antioxidant enzymes and protein.

Groups	Kidney MDA (µg/g tissue)	Kidney SOD (U/mg protein)	Kidney catalase (Katf)	Kidney GST (nmol/mg protein)	Kidney GSH (µg/ml)	Kidney Protein (mg/ml)
1	0.69±0.15	4.80±0.13	0.047±0.001	0.022±0.0013	6.802±0.2	1.678±0.03
2	0.48±0.09	4.40±0.14	0.050±0.0012	0.023±0.00129	6.956±0.1	1.96±0.028*
3	3.85±0.20*#	2.00±0.09*#	0.014±0.001*#	0.015±0.009*#	4.006±0.09*#	1.872±0.02*#
4	1.79±0.21*#	3.20±0.12*	0.030±0.004*	0.014±0.0012*#	4.64±0.2*#	1.632±0.02
5	0.67±0.17	4.40±0.10	0.040±0.003	0.021±0.001	6.594±0.1	1.596±0.014
6	2.16±0.15*#	2.80±0.76*#	0.025±0.005*#	0.018±0.001	4.994±0.1*#	1.354±0.015*#

1= Control, 2=Onion Only, 3=Cyanide Only, 4=Cyanide+300mg onion extract, 5=Cyanide+600mgonion extract, 6=Cyanide+Sodium thiosulphate. S= Serum, U= Urine, *p< 0.05 (compared to control), #p<0.05(compared to Cyanide+600mg onion extract).

Cyanide is a rapid-acting mitochondrial toxicant that inhibits cytochrome oxidase, thereby blocking the flow of electrons through complex IV to prevent oxidative metabolism (Jones et al., 2003) and enhance ROS generation at complex III (Chen et al., 2003).The damaging effects of cyanide on the tissue and organ could be mediated by cyanide ion (being a nucleophile) and its inhibition of mitochondria respiratory chain thus producing free radical such as superoxide anion (Mills *et al.*, 1996), resulting in lipid peroxidation. Thus some of the effects observed in cyanide treated rats could be mediated through attack of the generated ROS on some target organ and cells such as the, kidney. Also exposure to high levels of ROS leads to depletion in antioxidant levels in the animals resulting in oxidative stress. (Wu and Cederbaum, 2003). The results in this study also confirm that cyanide exposure increase malondialdehyde (a product of lipid peroxidation) concentration in the kidney treated rats.

The increased concentration of LPO products observed in the kidney of cyanide treated rats is also associated with decreased activity of scavenging enzymes such as catalase and superoxide dismutase in the kidney of these animals. A decrease in the activities of these enzymes can lead to the excessive availability of superoxides and peroxy radicals, which in turn generate hydroxyl radicals resulting in the initiation and propagation of LPO (Sacks et al., 1978). Earlier studies have shown that cyanide inhibit the antioxidant enzymes catalase, SOD, glutathione peroxidase (Kanthasamy et al., 1997), GST and reduces the level of GSH (Elsaid and Magedah, 2006).

Supplementation of onion extract and sodium thiosulphate to cyanide treated rats counteracts the increased levels of LPO products in the kidney. This decreased LPO is associated with increased activities of the antioxidant enzymes-catalase and SOD which was more pronounced in the 600mg/kg onion extract co- administered than in animals co-adminitered with 300mg/kg or 600mg/kg sodium thiosulphate. This indicates that treatment with onion extract is dose dependent and also more effective than treatment with sodium thiosulphate.

One of the antioxidants in the body is the reduced glutathione, a reducing agents in biological cells that provide a primary antioxidant defence against reactive intermediates of metabolism, drugs or carcinogens (Meister and Anderson.1983). The present study also shows a decrease in the non-enzymatic antioxidant glutathione (GSH) content and the antioxidant glutathione-s-transferase (GST) activity in the kidney of cyanide treated rats, these may further contribute to the increase in LPO observed in these animals. This is in agreement with earlier studies in which ingestion of cassava cyanide depletes blood glutathione. This depletion in glutathione status could be one of the mechanisms by which cyanide exerts its numerous toxicities (Mcmillian and Geervarghese, 1979). Cysteine and glutathione derivatives are major constituents of onion (Lancaster and Kelly 1993), the presence of these compounds in onion could increase the non-enzymatic antioxidant (GSH) and the detoxifying enzyme (GST). These compounds have been shown to participate in s-thiolation process which affects the structure and functions of proteins. Thus onion could help reduce the damaging effect of cyanide by increasing the body's glutathione level and GST activity, as cyanide has been shown to deplete whole blood glutathione (Okafor et al 2008). Onions are also rich in flavonoid and phenolic which possess antioxidant properties (Lanzotti 2006).

Decreased LPO, high increased GST and GSH observed in sodium thiosulphate supplemented rats could also be attributed to the sulphur contained in it. While that of onion extract to its rich sulfur containing active compounds in the form of cysteine derivatives. It is known that onion oil contains dialkyl disulfides (CH–S–S–CH_3, CH3–S–S–C_3H5, CH3–S–S–C3H7, etc.) and their oxides and thiols, which can trap electrons from other systems (Klanns-Dieter, 1983). Thus it prevents superoxide formation to a certain extent and scavenges many free radicals including hydroxyl radicals.Therefore the antidotal effect of onion on cyanide toxicity may be due to the fact that organosulphur contained in onion have antioxidant and detoxifying ability. These detoxifying effects are related to their ability to increase antioxidant defence system during toxicity (Ola-Mudathir et al, 2008).

The present study has demonstrated that administration of onion extract to rat may effectively prevent cyanide-induced nephro-toxicity in rat. This

effect is associated with increase in the activity of SOD and catalase, GST, and in the level of non-enzymatic antioxidant GSH in the kidney of the animals. Thus the antioxidant effect of this flavonoid and sulphur rich extract from *Allium* Cepa Linn is reflected in the present study. These results also suggest that onion extract may be useful nutritional antioxidants and its supplementation ameliorate the tissue damage or oxidative stress caused by cyanide. This effect is dose-dependent and is more pronounced than that of sodium thiosulphate.

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Some Aspects of the State-of-the-Arts in Biomedical Science Research: A Perspective for Organizational Change in African Academia

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Summary: In the biomedical sciences, there is need to generate solutions for Africa's health and economic problems through the impact of university research. To guide organizational transformation, the author here presents some aspects of the state-of-the-arts of biomedical science research in advanced countries using a perspective derived from the FASEB journal publications. The author examines the thirty three peer reviewed scientific research articles in a centennial (April 2012) issue of the FASEB Journal [Volume 26(4)] using the following parameters: number of authors contributing to the paper; number of academic departments contributing to the paper; number of academic institutions contributing to the paper; funding of the research reported in the article. The articles were written by 7.97±0.61 authors from 3.46±0.3 departments of 2.79±0.29 institutions. The contributors were classified into four categories: basic sciences, clinical sciences, institutions and centers, and programs and labs. Amongst the publications, 21.2% were single disciplinary. Two tier collaboration amongst any two of the four categories were observed in 16/33 (48.5%) of the articles. Three tier and four tier collaborations were observed amongst 7/33 (21.2%) and 3/33 (9%) of the articles respectively. Therefore 26/33 (78.7%) of the articles were multidisciplinary. Collaborative efforts between basic science and clinical science departments were observed in 9/33 (27.3%) articles. Public funding through government agencies provided 85 out of a total of 143 (59.5%) grants. The collaborative and multidisciplinary nature and government support are characteristic of biomedical science in the US where research tends to result in solutions to problems and economic benefits.

Keywords*:* Organizational Change, Biomedical Science, FASEB, Biomedical Research, Nigeria, Africa

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INTRODUCTION

Ezema (2010) records that Nigeria produces 148/351 or 42.2% of African Journals OnLine's listings, followed by South Africa's 67/351 or 19.1%, the rest of Africa contributing less. Of the 351 journals listed, 107 are in the field of medicine, while the rest are in other sciences and arts. Thus Nigerians are capable of setting a new pace for Africa in the field of biomedical science. Across the world, new patterns have set into biomedical science. These include multidisciplinary and collaborative research and state-of-the-arts utilization of cell biology and molecular biology (John, 2013) with supporting organizational changes (Roush, 1997; Service, 1999; Metzger and Zare, 1999).

To harness this trend for policy making in Nigerian universities and in other African countries, the author here presents a study of the peer-reviewed scientific publications in a centennial issue of the FASEB Journal.

MATERIALS AND METHODS

The FASEB Journal, Volume 26(4), April, 2012 is a publication (FASEB Journal (2012)) of the time-tested Federation of American Societies for Experimental Biology, established in 1912 and celebrating its centenary in 2012 (FASEB Centennial Home Page, 2012). Twenty six of the composite scientific societies are listed in a previous article examining the biomedical science techniques utilized in the articles (John, 2013). From BioxBio.com (2014) website information, the recent impact factors and cites of the FASEB Journal articles are in the Table 1 below.

Table 1. Four-year impact factors of the FASEB Journal

Year	Impact Factor (IF)	Total Articles	Total Cites
2013/2014	5.48	466	41104
2012	5.704	462	39540
2011	5.712	409	38304
2010	6.515	462	38538

In the FASEB centennial year, 2012, the topmost biomedical science journals had the following impact factors for 2011: Nature, 26.28; Cell, 32.4; Science, 31.20 and the FASEB Journal was #26 amongst the thousands of global English-language journals and had maintained a standard within this range for 5 years (Science TechBlog, 2012). The FASEB journal is described as "The Journal That Covers All The

Life Sciences And The Life of Science" (according to the slogan on the inside front cover of the issue studied) (FASEB Journal, 2012).

The thirty three peer reviewed scientific research articles in the April 2012 issue of the FASEB Journal (Volume 26(4): 1413-1763 were studied using the following parameters:

a) Number of authors contributing to the paper
b) Number of academic departments contributing to the paper
c) Number of academic institutions contributing to the paper
d) Funding of the research reported in the article

The frequencies were recorded for each parameter and were tabulated or presented as charts. The means, medians, and modes were generated from Microsoft Excel 2010 functions and the equality of these values for any parameter was used to indicate a uniform population of biomedical science studied and therefore to define the state-of-the-arts of biomedical science in the USA. These findings are used to recommend infrastructural and organizational development for Nigerian and other African institutions seeking excellence as centres of biomedical science research and innovation.

RESULTS

Collaborative Nature of Biomedical Science Research

Table 2 and Figure 1 show that the peer reviewed scientific research articles published in the issue of the FASEB Journal studied (Volume 26(4)) were written by an average of 7.97±0.61 authors from 3.46±0.3 departments of 2.79±0.29 institutions. Of the 33 articles, 2/33 was submitted from a single

Table 2. Some defining parameters of collaborative biomedical science research in the USA gathered from 33 peer reviewed research articles of the April 2012 issue of the FASEB Journal.

	No. of Authors	No. of Departments	No of Institutions
Mean±SEM	7.97±0.61	3.46±0.30	2.79±0.29
Median	7	3	3
Mode	7	2	1

Table 3. Types of departments contributing to the 33 peer reviewed research articles of the April 2012 issue of the FASEB Journal.

1.	Basic science departments	e.g. Department of Molecular and Cell Biology (Kemaladewi *et al.*); Department of Pharmacology and Toxicology (Zuo *et al.*); Department of Immunology (Dancyger *et al.*)
2.	Clinical science departments	e.g. Department of Anesthesiology and Intensive Care Medicine (Mirakaj *et al.*)
3.	Multidisciplinary departments	e.g. Department of Physiology, Anatomy, and Genetics (Levett *et al.)*
4.	Specialized research laboratories	e.g. Integrative Physiology of Exercise Laboratory (Huttermann *et al*); Cell Imaging Laboratory (Zhang *et al.*)
5.	Specialized centres	e.g. Center for Molecular Medicine and Genetics (Huttermann *et al.*); Center for Altitude, Space, and Extreme Environment Medicine (Levett *et al.*)
6.	Research institutes	e.g. Institute of Child Health (Levett *et al.*)
7.	Specialized research group	e.g. Computational Biology and Bioinformatics Group (Tal *et al.*)
8.	Specialized programs	e.g. Vision Science Program (Wang *et al.*); Program in Immunology and Infectious Diseases (Dancyger *et al.*)
9.	Clinics	e.g. Clinic of Anesthesiology, Intensive Care Medicine, and Pain Therapy (Mirakaj *et al.*)

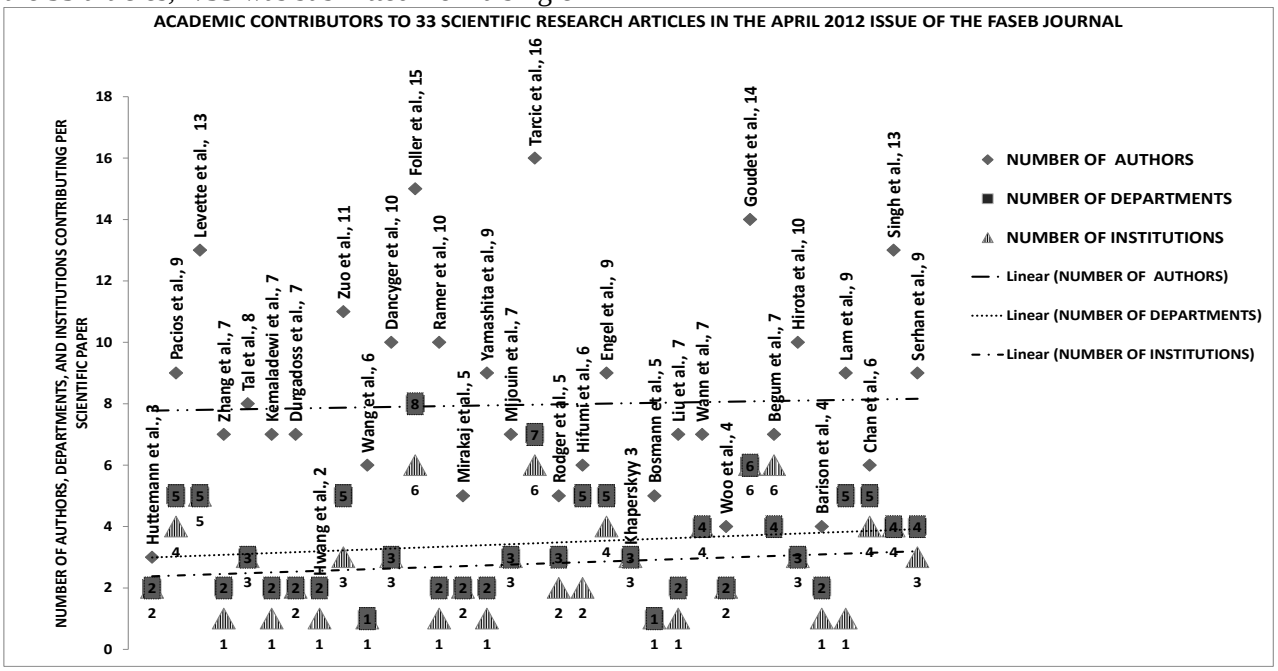

Figure 1. The 33 peer-reviewed research articles published in the April 2012 issue of the FASEB Journal showing some parameters that define collaborative efforts in the USA.

Table 4. Representations of basic science departments as contributors to the 33 peer-reviewed research articles in the April 2012 issue of the FASEB Journal.

BASIC SCIENCE DEPARTMENTS CONTRIBUTING TO 33 PEER REVIEWED SCIENTIFIC RESEARCH ARTICLES OF THE APRIL 2012 ISSUE OF THE FASEB JOURNAL	Huttemann *et al.*	Pacios *et al.*	Levett *et al.*	Zhang *et al.*	Tal *et al.*	Kemaladewi *et al.*	Durgadoss *et al.*	Hwang *et al.*	Zuo *et al.*	Wang *et al.*	Dancyger *et al.*	Foller *et al.*	Ramer *et al.*	Mirakaj *et al.*	Yamashita *et al.*	Mijouin *et al.*	Tarcic *et al.*	Rodger *et al.*	Hifumi *et al.*	Engel *et al.*	Khaperskyy *et al.*	Bosmann *et al.*	Liu *et al.*	Wann *et al.*	Woo *et al.*	Goudet *et al.*	Begum *et al.*	Hirota *et al.*	Barison *et al.*	Lam *et al.*	Chan *et al.*	Singh *et al.*	Serhan *et al.*
department of anatomy			*																														
department of applied chemistry																			*														
department of biochemistry												*																		*			*
department of biochemistry and molecular biology IV																				*													
department of biological regulation																	*																
department of biological sciences											*																						
department of cellular microbiology																													*				
department of cellular stress biology									*																								
department of chemistry																																	
department of craniofacial biology																								*									
department of environmental and molecular toxicology					*																												
department of human and molecular genetics									*																								*
department of immunology									*		*																						
department of microbiology																			*														
department of microbiology and immunology									*												*												
department of molecular and cell biology						*																											
department of neurosciences																									*								
department of pathology		*																				*									*		
department of pathology and laboratory medicine																					*												
department of pharmacology and toxicology									*																					*			
department of physics of complex systems																	*																
department of physiology												*															*						
department of physiology and pharmacology																												*					
department of physiology and medical physics																				*													
department of physiology, anatomy, and genetics			*																														
department of physiology, development, and neuroscience			*																														
department of systems biology																	*																
school of anatomy and human biology																								*									
school of engineering and material science															*																		
second department of anatomy															*																		
TOTAL BASIC SCIENCE DEPARTMENTS PER ARTICLE	0	1	3	0	1	1	0	0	5	0	2	2	0	0	2	0	3	0	2	2	2	1	0	2	1	0	1	1	1	2	1	0	2

department (Bosmann *et al*., Department of Pathology University of Michigan Medical School and Wang *et al.*, Vision Science Program, School of Optometry, University of California, Berkeley). Of the 33 articles, 10/33 were submitted from 2 departments within a discipline or between two disciplines. The remaining 21/33 articles were submitted by contributors from 3 or more departments within a discipline or across disciplines.

Contributing Departments

The types of departments contributing to the biomedical science research articles are listed in Table 3. Work published could be strictly disciplinary

Table 5. Representations of clinical science departments as contributors to the 33 peer-reviewed research articles in the April 2012 issue of the FASEB Journal.

CLINICAL SCIENCE DEPARTMENTS AND DIVISIONS CONTRIBUTING TO 33 PEER REVIEWED SCIENTIFIC RESEARCH ARTICLES OF THE APRIL 2012 ISSUE OF THE FASEB JOURNAL	Huttemann *et al.*	Pacios *et al.*	Levett *et al.*	Zhang *et al.*	Tal *et al.*	Kemaladewi *et al.*	Durgadoss *et al.*	Hwang *et al.*	Zuo *et al.*	Wang *et al.*	Dancyger *et al.*	Foller *et al.*	Ramer *et al.*	Mirakaj *et al.*	Yamashita *et al.*	Mijouin *et al.*	Tarcic *et al.*	Rodger *et al.*	Hifumi *et al.*	Engel *et al.*	Khaperskyy *et al.*	Bosmann *et al.*	Liu *et al.*	Wann *et al.*	Woo *et al.*	Goudet *et al.*	Begum *et al.*	Hirota *et al.*	Barison *et al.*	Lam *et al.*	Chan *et al.*	Singh *et al.*	Serhan *et al.*
department of anatomy with radiology																								*									
clinic of anesthesiology, intensive care medicine and pain therapy					*																												
department of anesthesiology and intensive care medicine														*																			
department of anesthesiology, perioperative and pain medicine																																	
department of endocrinology and diabetes																											*						
department of gastroenterology,hepatology, and endocrinology																																*	
department of internal medicine																																*	
department of medicine																								*									
department of neurology												*																					
department of neurosurgery																				*													
department of nursing																			*														
department of pediatrics																							*								*		
department of periondontics		3*																															
department of periondontology		*																															
division of cardiology				*																													
division of cardiothoracic surgery																														*			
division of colorectal surgery																														*			
division of gastroenterology																														*			
division of hematology and oncology																															*		
division of molecular and cellular neurosciences							*																										
division of neurodegenerative diseases												*																					
experimental and regenerative neuroscience																		*															
medical school university of Crete																											*						
Sackler school of medicine																		*															
second department of internal medicine													*																				
TOTAL CLINICAL SCIENCE DEPARTMENTS PER ARTICLE	0	4	0	1	1	0	1	0	0	0	0	2	1	1	0	0	0	2	1	1	0	0	1	2	0	0	2	0	0	3	2	2	0

but collaborative involving 2 or more departments from different institutions e.g., Pacios *et al*. was a paper contributed by a Department of Periondotics, University of Pennsylvannia, USA, a Department of Periondotics, Universitat Internacional de Catalunya, Spain; a Department of Periondontology, Peking University, China; and a Department of Periondontics, University of Medicine and Dentistry of New Jersey, USA plus a Department of Pathology, University of Pennsylvania, USA

Multidisciplinary Nature of Biomedical Science Research

There were 30 varieties of basic science titled departments (Table 4) amongst contributors to the 33 articles. Out of the 33 articles published, 12 articles did not list any of these basic science departments as contributors, 9 articles include one of these basic science departments as a contributor, and 12 articles list 2-5 of these basic science departments as contributors. In total, the mean number of basic science departments per research article was 1.15±0.2, the mode was 0, and the median was 1.

There were 25 varieties of clinical science titled departments (or divisions) (Table 5). Out of the 33 articles published, 17 articles did not list any of these clinical science departments as contributors, 8 articles include one of these clinical science departments as a contributor, and 8 articles listed 2-4 of these clinical

Table 6. Representations of institutes and centers as contributors to the 33 peer-reviewed research articles in the April 2012 issue of the FASEB Journal.

INSTITUTES AND CENTERS CONTRIBUTING TO 33 PEER REVIEWED SCIENTIFIC RESEARCH ARTICLES OF THE APRIL 2012 ISSUE OF THE FASEB JOURNAL	Huttemann *et al.*	Pacios *et al.*	Levett *et al.*	Zhang *et al.*	Tal *et al.*	Kemaladewi *et al.*	Durgadoss *et al.*	Hwang *et al.*	Zuo *et al.*	Wang *et al.*	Dancyger *et al.*	Foller *et al.*	Ramer *et al.*	Mirakaj *et al.*	Yamashita *et al.*	Mijouin *et al.*	Tarcic *et al.*	Rodger *et al.*	Hifumi *et al.*	Engel *et al.*	Khaperskyy *et al.*	Bosmann *et al.*	Liu *et al.*	Wann *et al.*	Woo *et al.*	Goudet *et al.*	Begum *et al.*	Hirota *et al.*	Barison *et al.*	Lam *et al.*	Chan *et al.*	Singh *et al.*	Serhan *et al.*
A. A. Martinos center for biomedical imaging																										*							
Beatrice Hunter cancer research institute																															*		
center for altitude, space, and extreme environment medicine						*																											
center for experimental therapeutics and reperfusion injury																											*						
center for human and clinical genetics	*																																
center for molecular medicine and genetics							*																										
center for neuroscience																																	*
Dana-Faber cancer institute																																	
Forschergruppe 923 institute fur pharmakologie und toxiologie												*																					
German center for neurodegenerative diseases												*																					
Hertie-institute for clinical brain research																																*	
Instituo de investigacion sanitaria del hospital clinico san carlos																				*													
instituo de neuroquimica de la UCM																										*							
institut de genomique fonctionelle			*																														
institute for sport, exercise, and health																										*							
institute national de lasante et de la recherche medical			*																														
institute of child health																	*																
institute of molecular biology and biotechnology													*																				
institute of toxicology and pharmacology			*																														
instutute for human health and performance	*																																
Liggins Institute																																	*
Loker hydrocarbon research institute																												*					
national research center for growth and development											*																						
research center for applied medical engineering																	*																
Sheba cancer research center					*																												
TOTAL INSTITUTES AND CENTERS PER ARTICLE	2	0	3	0	1	1	1	0	0	0	1	2	1	0	0	0	2	0	0	1	0	0	0	0	0	3	1	1	0	0	1	1	2

science departments as contributors. In total, the mean number of clinical science departments per research article was 0.82±0.18, the mode was 0, and the median was 0.

There were 25 varieties of institutes and centres (Table 6). Out of the 33 articles published, 17 articles did not list any of these institutes and centres as contributors, 10 articles include one of these institutes and centres as a contributor, and 6 articles listed 2-3 of these institutes and centres as contributors. In total, the mean number of institutes

Table 7. Representations of specialized programs and laboratories as contributors to the 33 peer-reviewed research articles in the April 2012 issue of the FASEB Journal.

PROGRAMS AND LABORATORIES CONTRIBUTING TO 33 PEER REVIEWED SCIENTIFIC RESEARCH ARTICLES OF THE APRIL 2012 ISSUE OF THE FASEB JOURNAL	Huttemann *et al.*	Pacios *et al.*	Levett *et al.*	Zhang *et al.*	Tal *et al.*	Kemaladewi *et al.*	Durgadoss *et al.*	Hwang *et al.*	Zuo *et al.*	Wang *et al.*	Dancyger *et al.*	Foller *et al.*	Ramer *et al.*	Mirakaj *et al.*	Yamashita *et al.*	Mijouin *et al.*	Tarcic *et al.*	Rodger *et al.*	Hifumi *et al.*	Engel *et al.*	Khaperskyy *et al.*	Bosmann *et al.*	Liu *et al.*	Wann *et al.*	Woo *et al.*	Goudet *et al.*	Begum *et al.*	Hirota *et al.*	Barison *et al.*	Lam *et al.*	Chan *et al.*	Singh *et al.*	Serhan *et al.*
cell and imaging laboratory			*																														
human biology																*																	
IFR 136 agents transmissibles et infectiologie																				*													
integrative physiology of exercise laboratory																			*														
Japan science and technology agency-core research for evolutional science and technology																										*							
laboratoire de chimie et biochemie pharmacologiques et toxocologiques																										*							
laboratoire de neurobiologie de la cognition								*																									
laboratory of signal transduction																										*							
labotatoire de pharmacologie et biochimie de la synapse																											*						
MatTek corporation																												*					
Meakins-Christie laboratories																	*																
Program in immunology and infectious diseases																													*				
protein purification core facility																			*														
section of molecular oncology and immunotherapy																																	*
sensory plasticity laboratory																	*																
skeletal biology laboratory																*																	
unite des interactions bacteries-cellules																		*															
unite mixte de recherche 7102																*																	
UR1282 infectiologie animale et sante public										*																							
vision science program																									*								
world class university neurocytomics program																							*										
accelrys SARI																					*												
cell and gene therapy research group				*																													
child and youth health														*																			
computational biology and bioinformatics																															*		
TOTAL PORGRAMS AND LABORATORIES PER ARTICLE	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	3	2	1	2	1	1	0	1	0	1	3	1	1	1	0	1	0	1

and centres per research article was 0.73±0.16, the mode was 0, and the median was 0.

There were 25 varieties of programs and laboratories (Table 7). Out of the 33 articles published, 14 articles did not list any of these programs and laboratories as contributors, 15 articles include one of these programs and laboratories as a contributor, and 4 articles listed 2-3 of programs and laboratories as contributors. In total, the mean number of programs and laboratories per research article was 0.76±0.14, the mode was 1, and the median was 1.

The collaborative activities were analysed in 4 tiers (Table 8): one tier collaboration amongst basic science departments, amongst clinical science departments, amongst institutes and centres, or amongst programs and special labs were observed in 7/33 (21.2%) publications. Therefore 21.2% of the studies were single disciplinary. Two tier collaboration amongst any two of the four categories were observed in 16/33 (48.5%) of the articles. Three tier collaboration amongst any three of the four categories were observed amongst 7/33 (21.2%) of the articles. Four tier collaboration amongst the four categories were observed in 3/33 (9%) of the articles. Therefore 26/33 (78.7%) of the articles were multidisciplinary involving two-tier, three-tier, or

Table 8. The 33 peer-reviewed research articles in the April 2012 issue of the FASEB Journal showing collaborations across four tiers of disciplines.

	Huttemann *et al.*	Pacios *et al.*	Levett *et al.*	Zhang *et al.*	Tal *et al.*	Kemaladewi *et al.*	Durgadoss *et al.*	Hwang *et al.*	Zuo *et al.*	Wang *et al.*	Dancyger *et al.*	Foller *et al.*	Ramer *et al.*	Mirakaj *et al.*	Yamashita *et al.*	Mijouin *et al.*	Tarcic *et al.*	Rodger *et al.*	Hifumi *et al.*	Engel *et al.*	Khaperskyy *et al.*	Bosmann *et al.*	Liu *et al.*	Wann *et al.*	Woo *et al.*	Goudet *et al.*	Begum *et al.*	Hirota *et al.*	Barison *et al.*	Lam *et al.*	Chan *et al.*	Singh *et al.*	Serhan *et al.*	
TOTAL BASIC SCIENCE DEPARTMENTS PER ARTICLE	0	1	3	0	1	1	0	0	5	0	2	2	0	0	2	0	3	0	2	2	2	1	0	2	1	0	1	1	1	2	1	0	2	
TOTAL CLINICAL SCIENCE DEPARTMENTS PER ARTICLE	0	4	0	1	1	0	1	0	0	0	0	2	1	1	0	0	0	2	1	1	0	0	1	2	0	0	2	0	0	3	2	2	0	
TOTAL INSTITUTES AND CENTERS PER ARTICLE	2	0	3	0	1	1	1	0	0	0	1	2	1	0	0	0	2	0	0	1	0	0	0	0	0	3	1	1	0	0	1	1	2	
TOTAL PORGRAMS AND LABORATORIES PER ARTICLE	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	3	2	1	2	1	1	0	1	0	1	3	1	1	1	0	1	0	1	
basic-clinical collaboration		*			*							*							*	*				*			*			*	*			9
1 tier collaboration	#							#	#	#					#	#						#												7
2 tier collaboration		&		&		&	&				&		&	&				&			&		&	&	&	&		&	&		&		16
3 tier collaboration			@		@							@					@		@									@					@		7
4 tier collaboration																				$							$				$				3
																																		TOTALS

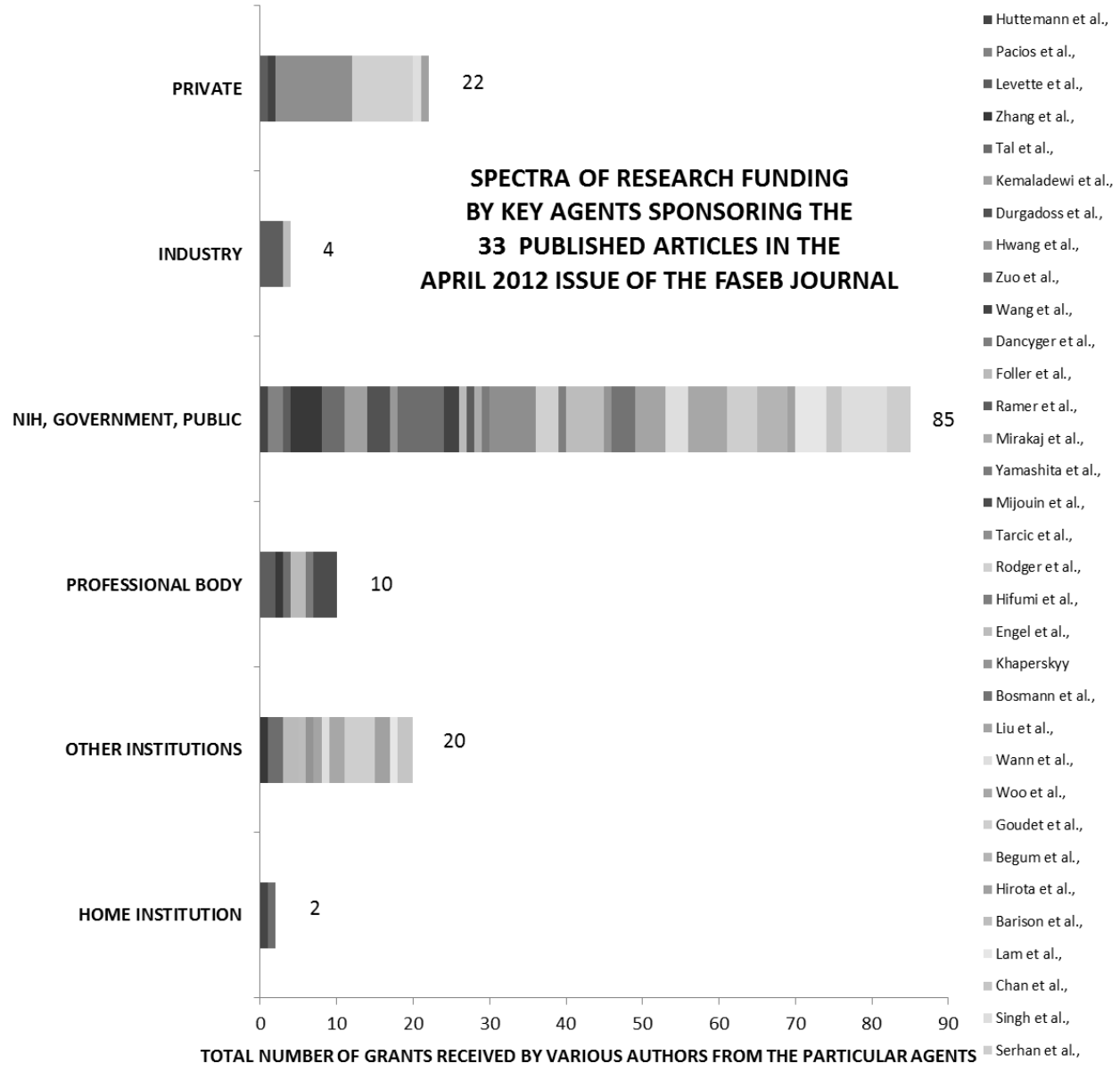

Figure 2. A Perspective of funding of biomedical science research in the USA from articles published in the April 2012 issue of the FASEB Journal.

Table 9. Number of grant support per research article in the April 2012 issue of the FASEB Journal

Authors	Total Funding
Huttemann *et al.,*	2
Pacious *et al.,*	2
Levette *et al.,*	7
Zhang *et al.,*	6
Tal *et al.,*	4
Kemaladewi *et al.,*	3
Durgadoss *et al.,*	3
Hwang *et al.,*	1
Zuo *et al.,*	9
Wang *et al.,*	3
Dancyger *et al.,*	0
Foller *et al.,*	5
Ramer *et al.,*	1
Mirakaj *et al.,*	1
Yamashita *et al.,*	2
Mijouin *et al.,*	3
Tarcic *et al.,*	16
Rodger *et al.,*	11
Hifumi *et al.,*	1
Engel *et al.,*	7
Khapersky *et al.,*	2
Bosmann *et al.,*	3
Liu *et al.,*	5
Wann *et al.,*	5
Woo *et al.,*	7
Goude *et al.,*	8
Begum *et al.,*	4
Hirota *et al.,*	4
Barison *et al.,*	0
Lam *et al.,*	5
Chan *et al.,*	4
Singh *et al.,*	6
Serhan *et al.,*	3

four-tier collaboration. Collaborative efforts between basic science and clinical science departments were observed in 9/33 (27.3%) articles.

Funding of Research

A total of 143 grants produced the 33 published research articles. The major categories of sponsors of the research articles are shown in Figure 2. Public funding directly through government agencies provided 85 grants out of the total of 143 grants (59.5%). Private funding provided 22/143 grants (15.39%), other institutions provided 20/143 grants (14%), and professional bodies provided 10/143 grants (7%). The Industry contributed 4/143 grants (2.8%) and home institutions contributed 2/143 grants (1.4%). Table 9 shows the number of funding support per research article. The list is according to stated sponsors. Two papers did not list sponsors but were from specialized institutes, the institutes being government owned. Number of grants per article ranged from 1-16, the mean being 4.3, the mode being 3, and the median being 4.

DISCUSSION

The study of the 33 peer-reviewed research articles of the April 2012 issue of the FASEB Journal shows that each paper was produced by collaborative efforts of authors from separate departments within the same discipline or between different disciplines. About seven authors and 3 departments usually contribute to such publications delivering various types of evidence that help to answer the questions being asked in the investigations.

For **number of authors**: the mean was 7.97±0.61, the median was 7 and the mode was 7 therefore authors who wish to write good state-of-the-arts biomedical science research reports can use this popular trend as a guide: to *involve about half a dozen collaborators/co-authors/experts*.

For **number of departments** and number of institutions the mean, mode, and median did not coincide reflecting a definite diversity using this parameter. The *multiplicity of departments and institutions* (Table 2, Figure 1) can be another guide for researchers embarking on a study. While only two studies were submitted by contributors from a single department, 31/33 articles were submitted by contributors from 2 or more departments within a discipline or across disciplines and within an institutions or across institutions.

The contributing departments include traditional basic and clinical science departments [Table 3,(1.) and (2.)] as well as a variety of diverse, specialised, and innovative research outfits [Table 3, (3.)–(9.)] showing a shift from traditional subject departments in various cases. In Nigerian and other African universities, while traditional basic and clinical science subject departments are needed as important subject teaching turfs, there may be need for *innovation of numerous and diverse specialized research outfits with specific developmental goals*, similar to or different from those we see in Table 3. This may be a significant part of any effort towards specific research that serves national health and economic needs more. The present analysis revealed co-existence of departments with traditional disciplines and departments that make a diversion from traditional turfs in developed countries. Amongst the contributing departments were basic science departments, clinical science departments, multidisciplinary departments, specialized research laboratories, specialized centers, research institutes, specialized groups, and clinics as stated in the Results (Tables 3, 4, 5, 6, and 7). This reflects **organizational innovations** to facilitate research interests. Specially organized research activity can

also attract specific sponsors that have a focus on particular interests.

Infrastructural and organizational deficiencies are well recognized in Nigeria and other parts of Africa (John, 2009, 2010, 2012a). As seen in the results, collaboration in developed countries goes across national borders and this aspect may also need to be better addressed in African sub-regions. **Pan-African research** collaborative efforts may help offset the hindering aspects of poverty and low funding across Africa and provide a platform for combining resources and a potential for more successful research.

Beyond Pan-African collaborations, Nigerian and other African researchers can find long-term support in the sophisticated research outfits of advanced countries in Europe and Asia as well as in the USA and Canada. Noteworthy amongst the 33 articles is collaboration that extends *beyond continental boundaries*. For example, Pacios *et al*., was a collaboration between periondontics researchers in the USA, Spain, and China and Tarcic *et al*., was a collaboration by multidisciplinary researchers in USA, Israel, and Greece. Therefore, for Africans also, **global support** through the extension of roles and sharing of topic expertise, technical knowhow, sophisticated research procedures, expensive and high-maintenance equipment and infrastructure, and materials and supplies can allow African researchers to embark on and complete ambitious and profitable investigations which they cannot afford to do on their own.

While research funding in the USA appears to be largely dependent on the government (Figure 2), this kind of support may not be possible in African nations that do not enjoy lasting political stability. Seventeen years ago, the author of this article was a beneficiary of a grant of $10,000.00 from the Organization of African Unity Scientific and Technical Research Committee, a well-organized, high achieving organization that gave out research grants regularly to individual African scientists from different fields. This committee no longer exists because the OAU was disbanded on July 9th, 2002 and replaced by the African Union. The African Union has a Human Resources, Science, and Technology Commission but had, by 2012, only been able to make two calls for award of research grants, one in 2012 and for 3 specific topics: post-harvest and agriculture, sustainable energy, and sanitation (African Union, 2012). Thus biomedical science was not yet one of its out reaches. Where once Africans have tried to set up appropriate research collaborations, political instability or changes may have been the bane of such efforts. Nigerian and other African scientists indeed need to find ***sustainable means*** of continuous scientific collaborations and funding which cannot be disturbed by any political climate and which is resistant to negating economic factors. The professional bodies such as The West African Society for Pharmacology (WASP/SOAP 2012) and The West African Health Organization (WAHO 2012) may need to generate private funds, independent of governments, to distribute to individuals or groups competitively for research.

Tables 4-7 show that *biomedical science research* is being done by diverse departments which can be grouped into: basic science departments, clinical science departments, institutes and centers, and specialized programs and laboratories. This can only be possible if there are basic scientists working in clinical departments, institutes, and specialized programs and laboratories as well as if there are physician scientists who not only work in clinics but actually do bench research like the basic scientists do. The author of this article was a PhD Pharmacologist, working for a few years as an assistant professor in a clinical department, a neonatology unit, in the USA. This is a common occurrence in the USA. Of the 33 articles studied in this report, twelve articles did not list a basic science department as contributor therefore the biomedical science research was done beyond basic science turf. In the USA, for the sake of collaborative, multidisciplinary research supported by grant awarding bodies, efforts have been made to reduce departmental territoriality and promote **employment of a diversity of researchers** as needed in respective research outfits (Roush, 1997; Service, 1999, Metzger and Zare, 1999).

Apart from the departmental collaborations within disciplines, we see departmental collaborations across disciplines. Table 8 shows that only 21.2% of the publications were from contributors from a single discipline and 78.7% of the publications were by multidisciplinary collaborations. This reflects the fact that *much of biomedical science research is* ***topic-based research*** (cancer, HIV-AIDS, effects of hypoxia, lung function, etc.) rather than subject based (anatomy, physiology, biochemistry, pathology, microbiology, paediatrics, surgery, etc.). Research thus appears to be done to find solutions to prevailing societal health problems and to support existing clinical needs or to produce marketable services and products according to the aims of sponsors. The involvement of subject disciplines in any study appears to be as needed and without discrimination or rigidity of subject boundaries. Two-tier collaboration was most common (48.5%) and four tier collaborations were least common (9%) indicating that *collaboration is not an end but a means*. About a quarter (27.3%) of collaborations were between basic science and clinical science departments and the

majority of collaborations extend the diversity amongst institutes and centers and specialized programs and laboratories.

This analysis of research funding shows that the home institutions possibly contributed the least funds (1.4%) to the research work emanating from themselves. **Abundant local funding** (59.5%) is from organized government functionaries and their grant awarding bodies. The involvement of specially organized governmental research funds awarding organizations that award research money routinely and competitively shows that *much of the research is actually guided towards national interests.* In Africa, more government involvement in research direction and funding is necessary to obtain good long-term solutions that meet local health and economic needs. Since *academic freedom* is an important aspect of research, researchers also need to be able to obtain funding for their own *curiosity research* interests different from those directed by government interests. From these results, the availability of funding from private sectors (15.39%), other institutions (14%), and professional bodies (7%) makes it possible for scientists to seek funding from preferred sponsors according to scientists' research interests or research focus. Interestingly, industry appears to produce 2.8% of the total funding in these publications.

The apparent low contribution by industry to funding of published biomedical science as seen in this investigation may reflect corporate and private-interest policies of discretion and non-publication of profitable research, powerful research, secret research, or questionable research (Resnik, 1998, 2006a; Levine, 1988; Krimsky 2003). It is well recognized that: **"**Modern science is big business. Governments, universities, and corporations have invested billions of dollars in scientific and technological research in the hope of obtaining power and profit. For the most part, this investment has benefited science and society, leading to new discoveries, inventions, disciplines, specialties, jobs, and career opportunities. However, there is a dark side to the influx of money into science. Unbridled pursuit of financial gain in science can undermine scientific norms, such as objectivity, honesty, openness, respect for research participants, and social responsibility" (Resnik, 2006b). Apart from the issue of trade secrets, some of industry sponsored research is clinical research and may be held from publication because of protection of confidentiality (Shamoo and Resnik, 2003) required by the Privacy Rule of the Healthcare Information Portability and Accountability Act (HIPAA). Of course, amongst these articles studied, there may be no classified research even though it is known that: "The U.S. government spends more on military and national security research than all other types of research combined" (Kintish and Mervis 2006).

Collaborations with advanced countries can help to gain the exposure needed to attract significant **foreign funding.** As mentioned above, amongst the studies analysed, Pacios *et al.* (2012) was from collaborations between the USA, China, and Spain and Tarcic *et al.* (2012) was from collaborations between the USA, Israel, and Greece. The former paper was sponsored by two US grants from the US National Institute of Dental and Craniofacial Research. The latter paper was sponsored by 3 grants from the US National Cancer Institute, plus grants from The European Commission, The German-Israeli Project Cooperation, The Israel Cancer Research Fund, the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, the Kekst Family Institute for Medical Genetics, the Kirk Center for Childhood Cancer and Immunological Disorders, the Women's Health Research Center funded by the Bennett-PrItzker Endowment Fund, the Marvelle Koffler Program for Breast Cancer Research, the Leir Charitable Foundation, the M.D. Moross Institute for Cancer Research, and The Susan G. Komen Foundation while two of the contributors' professorial chairs were endowed by sponsors: Harold and Zelda Goldenberg and Henry J. Leir.

SPECULATION

In advanced countries such as the USA, biomedical science research, as published in the 33 peer reviewed research articles in a centennial (April 2012) issue of the FASEB Journal is characterized by ***collaborative efforts*** amongst about 7 investigators, typically from 2 or more departments of 2 or more institutions which may include different countries. Biomedical science research in the USA is typically ***multidisciplinary*** involving 2 or more basic medical sciences, clinical sciences, or both or innovative research organizations. Biomedical science research in the USA is heavily ***funded by government*** agencies. While biomedical science in the USA may not be a model in every respect (John, 2011, 2012b), these factors may benefit Nigerian and other African biomedical scientists and researchers as they try to forge ahead for better results in biomedical science. *Well-funded, collaborative, and multidisciplinary organization of research projects may be a means to national progress and development.*

CONCLUSION

From the present study, ideas arising for organizational change towards transformative academic biomedical science research in Nigeria and other African countries are: a) cross-territorial topic-based in addition to territorial subject-based biomedical science research; b) generation of novel

academic research outfits; c) involving several diversified experts from different departments in a study; d) involving collaborating departments, institutions, and countries in particular studies; e) sharing of organizational resources to support transformative research; f) sustainable means of continual funding and resource generation; g) structures for sufficient local funding, especially from governments; and h) global support. These are, perhaps, some of the important tasks for the present day Nigerian, indeed African, academic in the field of biomedical science to accomplish or improve and to consider in faculty development and organizational change exercises.

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Short Communication

Intestinal Ischaemia-Reperfusion Injury and Semen Characteristics in West African Dwarf Bucks

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Summary: Increasing production of goats takes their reproductive potential and fertility, into consideration. Gastrointestinal obstructive lesions can set up an intestinal ischaemia-reperfusion. Testicular torsion is an established cause of testicular damage and infertility and is a form of ischaemia-reperfusion injury. This study investigates the effect of intestinal ischaemia-reperfusion (IIR) injury on semen characteristics in WAD bucks. Six healthy adult male WAD goats were divided into two groups of three, a control and IIR group, one hour ischaemia and two hours of reperfusion were achieved in the intestinal ischaemia-reperfusion (IIR) group after all goats underwent a laparotomy. Semen collection was done using the electro-ejaculator method pre-operatively and weekly for four weeks post-operatively. The semen concentration, percentage of normal sperm cells, abnormal sperm cells and percentage abnormality were evaluated. In control animals, there was an increase in semen concentration postoperatively followed by a decrease whereas in IIR animals, a decrease was observed postoperatively till the 4th week. Total normal sperm cells decreased postoperatively and then increased to preoperative levels whereas a decrease was seen in IIR animals till the 3rd postoperative week. Abnormalities in sperm cells, normal head without tail, normal tail without head, bent mid-piece, curved mid-piece and rudimentary tail were all increased by the 4th week in IIR group though the total number of abnormal cells was observed to have decreased. The main effect of intestinal ischaemic-reperfusion injury on the semen characteristics of WAD goats is an increase in abnormalities with an adequate quantity of semen. Many of the abnormalities involved midpiece and tail abnormalities which are very vital to propulsion and may cause an inability of the sperm cells to fertilize. This hitherto silent phenomenon in farm animals may be the reason for iatrogenic causes of infertility.

Keywords*:* Intestinal Ischaemia-Reperfusion injury; semen charactieristics; West African Dwarf bucks.

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INTRODUCTION

The small ruminant industry in Nigeria represents a very important national resource contributing considerably to the national wealth, supply of protein, raw materials for industries and for festive and religious occasions (Remi-Adewumi et al., 2004). In Nigeria, goats are the most numerous of all types of livestock numbering about 27.6 million (Federal Office of Statistics, 1986). The animals are primarily for meat production (Hassan and Ciroma, 1992). Research attempts at improvement of reproductive potential of these small ruminants have made assessments of semen and semen characteristics necessary. Reproductive ability in the male comprises the production of semen containing normal spermatozoa (quality) in the adequate number (quantity), together with the desire and ability to mate (Oyeyemi and Ubiogoro, 2005).

Increasing incidence of gastro-intestinal emergencies in sheep and goat are due to difficulties which include methods of husbandry, increased pressure of land utilization in urban areas, lack of enforcement of legislation on land use, the increasing use and poor waste disposal of nylon bags used for food storage (Ghurashi *et al*., 2009). Bowel obstructions, especially strangulating types are medical and surgical emergencies with grave consequences due to hypovolemia and septic shock secondary to devitalization of the intestinal wall (Eyarefe *et al.,* 2011). In Nigeria, intestinal conditions rank 8th of 11 categorized surgical conditions and made up 7% of surgical cases presented to Veterinary Teaching Hospitals and clinics most of which were strangulating obstructive conditions such as intussuception, volvulus and torsion which were recorded for large and small animals. Many of these surgical problems create ischaemia in the bowels and an attempt to correct the problems set up an ischaemia-reperfusion situation.

Intestinal Ischaemia-reperfusion injury generally stems from interruption of blood flow within the cranial mesenteric artery or vein and leads to small intestinal hypo-perfusion and a mortality rate of approximately 70% (Tendler, 2003). Testicular ischaemia-reperfusion is an established cause of testicular damage and infertility (Shalaby and Afifi, 2008). This study investigates the effects of intestinal

ischaemia-reperfusion on semen characteristics and fertility.

MATERIALS AND METHODS

Six male West African Dwarf goats aged one year and weighing 13.5 ± 0.71kg were used for this study. They were divided into two groups, A (control) and B (IIR). Goats were fasted overnight, sedated with Xylazine (0.05mg/kgbwt) and an inverted L block of the right flank was done using 2% lignocaine (6mls). Control animals were sham operated by doing a laparotomy and closing up while the IIR animals had a laparotomy as well as intestinal ischaemia-reperfusion (IIR) injury. IIR was achieved by clamping the superior mesenteric artery for one hour and declamped to achieve reperfusion for two hours.

Procedure

Surgical site was prepared by shaving and washing with soap, water and methylated spirit. The skin and abdominal muscles were incised to expose the intestines which were exteriorized and the cranial mesenteric artery was located as it bifurcates from the aorta and clamped. Ischaemia was assessed as a paleness and bluish colouration of the intestines as well as loss of pulsation. Reperfusion was assessed as return of pulsation and change in colour of the intestines. Following reperfusion, the laparotomy was closed in three layers. Semen was collected by the electro-ejaculator method and examined macroscopically and microscopically according to Oyeyemi et al (2008).

Statistical Analysis

Data are presented as Mean ± SEM. Analysis was done using ANOVA, Duncan multiple comparison test and Student t-test. $p< 0.05$ is significant.

RESULTS

Sperm concentration decreased significantly from the 1st week following surgery and continued to decrease till the 4th week in IIR group (Table 1) while in control animals, did not show any decrease till the 4th week postoperatively. The normal sperm cells decreased in both IIR and control group of animals from the 1st week till the 2nd week and then increased (Table 1). The total abnormal cells in IIR group were higher significantly at the beginning and the end of the study, ie preoperatively and at week 4 in comparison with control animals (Table 1). Preoperatively, there appeared to be higher numbers of all semen abnormalities except CM and CT in IIR group than control group (Table 2). The first postoperative week (Table 2), there appears to be an increase in sperm abnormalities in the control group while the IIR group abnormality remained unchanged. The second postoperative week, except for CT, all abnormalities in the control animals appear to be increased than the IIR group (Table 2). The third postoperative week, all sperm abnormalities except LT have increased in IIR group (Table 2) whereas in the control group, they have either decreased or remained unchanged. The sperm abnormalities in the 4th week in IIR group are all significantly increased except for LT while those of control animals have all returned to preoperative levels (Table 2).

DISCUSSION

Testicular injury following torsion includes nonspecific damage induced by ischemia per se in addition to damage caused by reperfusion, so it is called ischemic-reperfusion (I/R) injury (Shalaby and Afifi, 2008). Gastro-intestinal emergencies in WAD goats can usually set up an obstructive surgical condition leading to the establishment of an ischaemia-reperfusion injury and the remote effects of intestinal IR have also been attributed to the ability of the intestine to serve as a generator for ROS and its unique position in the gastro-intestinal system in

Table 1: Sperm Concentration, Normal Sperm count and Total Abnormal Sperm Count before and following IIR in WAD goats.

	Sperm Concentration ($X10^9$ /ml)		**Normal Sperm Count (%)**		**Total Abnormal Sperm Count (%)**	
	Control	IIR	Control	IIR	Control	IIR
Preoperative	205±3.5	215±0.9	88.0	86.4	12.0	13.6
1week postop	215±2.8	205±1.2*	86.4	85.5	13.6	14.5
2weeks postop	200±2.2	215±6.5*	84.6	85.0	15.4	15.0
3weeks postop	200±2.1	180±3.5 *	86.4	85.2	13.6	14.8
4weeks postop	170±2.3	150±2.1*	88.0	86.6	12.0	13.4

*$p<0.05$

Table 2: Percentage Sperm Abnormality before and following IIR in WAD goats.

	NHWT		**NTWH**		**BM**		**CM**		**RT**		**BT**		**CT**		**LT**	
	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR
Preoperative	0.7	1.2	0.7	1.2	2.7	2.8	2.9	1.8	0.3	0.7	1.7	2.6	2.6	2.4	0.3	0.7
1week postop	1.3	1.5	0.9	0.7	2.6	2.7	1.8	2.4	0.9	0.7	3.0	3.3	2.4	2.2	0.3	0.7
2weekspostop	1.5	1.0	1.2	1.5	2.7	2.4	2.7	2.3	1.2	1.5	3.5	2.5	2.2	2.8	0.2	0.6
3weekspostop	0.6	1.5	1.5	1.3	2.6	2.8	2.6	3.3	1.5	1.3	2.4	2.0	2.2	2.4	0.2	0.2
4weekspostop	0.7	1.3*	1.3	1.5	1.7*	2.4	2.2	2.2	1.3	1.5*	2.0	2.2*	2.0	2.0	0.7	0.3

NHWT- Normal Head without Tail; NTWH-Normal Tail without Head; BM-Bent Middle piece; CM-Curved Middle piece RT-Rudimentary Tail; BT-Bent Tail;CT- Curved Tail; LT-Looped Tail; C- Control group; IIR-Intestinal Ischaemia Group.
*$p<0.05$

distributing its metabolites around the entire body which lends credence to authors (Ogbuewu *et al*., 2010) who found that excessive generation of ROS by abnormal spermatozoa & contaminated leucocytes is one of the few defined aetiologies for male infertility. The increased sperm abnormalities observed in this study around the fourth week may be due to pathophysiologic mechanisms that underlie testicular ischemic injury and germ cell death (Shalaby and Afifi, 2008) as due to a decrease in the blood flow which leads to reduction in oxygen supply relative to metabolic demands with subsequent depletion of the stored cellular energy and accumulation of toxic metabolites as lactic acid, hypoxanthine and lipid peroxides. There are reports (Franca *et al*., 1999) of spermatogenic cycles of 10.6 ± 0.5days in goats and a total spermatogenic cycle of 4.5cycles of seminiferous epithelium and lends credence to the effects of increased sperm abnormalities observed about the fourth postoperative week of this study.

The increased bent mid-piece and curved mid-piece abnormalities observed in the IIR group may be due to lipid peroxidation which is said to occur in IIR (Ogbuewu *et al*., 2010). Decreased semen concentration observed in the control group is less than that observed in IIR group and since a critical level of semen concentration is desirable for fertility as reported (Oyeyemi and Ubiogoro, 2005), the effect of IIR may be seen as a decrease beyond acceptable levels. This may well be the challenge that IIR poses in animals used in production. The remote effects of IIR need to be considered in cases of iatrogenic causes of infertility to establish a robust health determination when acquiring animals for a breeding program where history of gastrointestinal incidents may carry little significance until breeding problems occur.

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