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Original Article

OCULAR FINDINGS IN CHILDREN WITH CEREBRAL PALSY ATTENDING A TERTIARY HOSPITAL IN NORTH CENTRAL NIGERIA

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Abstract

Cerebral palsy is commonly associated with ocular abnormalities which often impact on their development and education. There is paucity of studies on this in Nigeria. We decided to study/ determine the prevalence of ocular abnormalities among children with cerebral palsy that attended the neurology clinic of University of Ilorin Teaching Hospital, Nigeria (UITH). A cross sectional survey of children with cerebral palsy presenting at Pediatric neurology clinic of UITH between January and December 2012 was done. Age appropriate visual acuities were checked, ocular examination was done with hand held Slit Lamp and Cycloplegic refraction and fundoscopy done with indirect Ophthalmoscope. Thirty - seven (42.5%) of 87 subjects seen within that period presented for ocular assessment. There were 24 males (64.9%) and 13 females (35.1%) with a male female ratio of 1.8:1. The age ranged between 6-168months with a mean of 50.11± 46.51months. Thirty- three (89.2%) were delivered full term while 4(10.8%) were preterm. Spastic cerebral palsy accounted for 45.9% of all the cases seen. Strabismus was found in thirteen subjects (35.1%). This was esotropia in 7(54%) and exotropia in 6 (46%) subjects. Refractive error was found in 32(86.5%) subjects and Cortical Visual Impairment (CVI) in 28(75.7%) subjects CVI, refractive errors and strabismus are the most common ocular findings in children with Cerebral Palsy in Nigeria. Early detection and early intervention is important to achieve best developmental and educational attainment. Multidisciplinary approach involving Neurologists, Ophthalmologists, and Physiotherapist is paramount in managing these children from the time of diagnosis.

KEY **WORDS: Ocular findings, Cerebral palsy, Cortical Visual Impairment, Strabismus**

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Introduction

Cerebral palsy is a term used to describe a spectrum of deficits of muscle tone and posture resulting from damage to the developing nervous system (Arnoldi, Pendarvis and Jackson et al 2006:97-107). Though thought to be a motor disorder, it can be associated with disorders of the sensory pathway (Arnoldi et al 2006:97-107).

The damage to the motor control in the developing brain can occur during pregnancy, perinatal or postnatal life. The resultant limitation in movement and posture can be accompanied by seizure disorder, abnormal muscle tone, dysarthria, sensory impairment, mental retardation and learning disabilities (Rosenbaum, Paneth and Leviton et al 2006:8-14). There are four main types, Spastic, ataxic, dyskinetic/athetoid and mixed cerebral palsy. The spastic type can be further classified into Spastic hemiplegia, spastic diplegia and spastic tetraplegia types. Beckung, Hagberg and Uldall (2008: e187-92) classified them as Spastic unilateral cerebral palsy, Spastic bilateral cerebral palsy and dyskinetic cerebral palsy and ataxic cerebral palsy. In the spastic type there is stiffness and movement difficulties, in ataxic form there is a disturbance of sense of balance and depth perception, while in the athetoid type there are involuntary and uncontrolled movements. Walking ability in children with cerebral palsy has been found to be significantly related to the type of cerebral palsy, IQ level, presence of active epilepsy and severe visual or hearing impairment (Beckung et al 2008: e187-92).

Flagnagan, Jackson and Hill (2003:493-9) while studying seventy-six children with visual impairment recruited from multiple sources found that 79% of them had medical problems, 33% of this was from cerebral palsy.

Also disorders of visual function are common findings in children with cerebral palsy (Ipata, Cioni and Bottai1994:95-203; Lagunju and Oluleye 2007:71-5; Ghasia, Brunstrom, Gordon et al 2008:572-80). The ocular abnormalities include strabismus, refractive errors, visual impairment, nystagmus and optic atrophy. Lagunju et al (2007:71-5) in Ibadan found that 50% of ocular abnormality in forty-two cerebral palsy children with ocular abnormality was due to strabismus, 47.7% cortical visual impairment, 4.8% refractive error and 9.5% nystagmus. Other ocular features that have been reported include cicatricial

retinopathy of prematurity and cortical blindness (Pennefather and Tin 2000:78-81). It has actually been found to be a risk factor for lack of development of object vision defined as ability to recognize faces or hand-held toys (Chen, Weinberg and Catalano 1992:575-8). Li, Yu and Zhang et al (2012:100) found that 9-30% of his cerebral palsy patients had visual impairment.

Disorders of visual function in cerebral palsy is often due to damage to central visual pathway (Guzzetta, Mercuri and, Cioni 2001:115-9; Sasmal, Maiti and Mandal 2011:318-323). This is because chronic hypoxia to the brain usually causes damage to the visual cortex. Visual function is related to cognitive, motor and emotional development since children learn to move, talk and do many things they see others doing. Hence a child with cerebral palsy that is also blind/visually impaired pose a greater challenge in terms of management and rehabilitation. Early detection and treatment of ocular problems will therefore enhance management in this group of children. This research is therefore aimed at determining the prevalence and ocular abnormalities among children with cerebral palsy that are attending the neurology clinic of UITH.

Materials and methods

A cross sectional survey of all children with cerebral palsy who attended the neurology clinic of University of Ilorin Teaching Hospital (UITH) between January and December 2012 were recruited into the study via phone calls in the year 2013. Ethical Approval was obtained from the University of Ilorin teaching Hospital Health Research Ethics committee. Informed consent was obtained from the parents/care givers of the children after explaining the purpose of the study/examination to them. Detailed history of their demographic data, pregnancy and birth history were obtained from parents or guardians to find the possible cause of cerebral palsy; type of cerebral palsy was obtained from their history and

examination of hospital records and documented in a semi-structured questionnaire.
Age-appropriate visual acuity assessment of respondents was carried out. Cover-Uncover test, Hirschberg and modified Krimsky test were used to check for strabismus. Extraocular muscle movements in the nine positions of gaze were assessed. The anterior segment was examined with the Keeler PSL classic hand held slit lamp. Cycloplegic (atropine) refraction was done using the retinoscope after which Posterior segment examination was done with indirect ophthalmoscope to check for optic atrophy and any other posterior segment lesions.

Spectacles were prescribed for those with refractive errors and those with strabismus or other treatable conditions were offered treatment. Data was analyzed using SPSS 16 statistical package. Variables were generated. P-value was set at less than 0.05.

Results

Thirty-seven (42.5%) subjects presented for ocular assessment out of a total of 87 children with cerebral palsy seen in 2012. There were 24 males (64.9%) and 13 females (35.1%) with a male female ratio of 1.8:1 (Table 1). Their ages ranged from 6-168months with a mean of 50.11± 46.51months. Thirty- three (89.2%) were delivered full term while 4(10.8%) were preterm. The birth weight was known in 19 (51.4%) cases with low birth weight i.e ≤ 2kg found in 4(21.1%) children. Twenty –one (56.8%) had birth asphyxia, 11(29.7%) did not while in 5(13.5%) it was unsure. Three (8.1%) had history of oxygen administration. History suggestive of encephalitis/meningitis was recorded in 4(10.8%) and hypoglycaemia in 2(5.4%) of subjects.

Table 1: Age / Sex distribution

AGE(Months)	Sex		NUMBER (%)
	Male	**Female**	
0-11	3	1	4(10.8)
12-60	13 `	10	23(62.2)
84-120	3	2	5(13.5)
132-180	5	-	5(13.5)
	24	**13**	**37(100)**

Spastic cerebral palsy accounted for 45.9% cases seen (Table 2). Majority (91.9%) of the children had delayed developmental milestones.

Vision assessment was CSM (Central Steady Maintained) in 24(64.9%) out of which 6(25%) on further assessment with LEA paddle appreciated 0.5 and 9.9cpm at 50mm. 13(35.1%) had visual acuity assessment by LEA symbols (Table 3). Cerebral visual impairment was found in 28(75.7%) of subjects.

Table 2: Types of Cerebral Palsy

Type of cp	Frequency	Percentage
Spastic	17	45.9
Hypotonic	6	16.2
Athetoid	2	5.4
Mixed	2	5.4
Ataxic	1	2.7
Uncharacterized	9	24.3
Total	**37**	**100.0**

Table 3: showing vision Assessment in patients with Cerebral Palsy

VA	Number	%
>6/18	2	5.4
≤ 6/18- 3/60	3	8.1
< 3/60-LP	3	8.1
NPL	5	13.5
CSM	24	64.9
Total	**37**	**100.0**

Seven (18.9%) of respondent were able to do the draw a man test for IQ assessment. Of these, one with 73% score was a slow learner, one with 60% score had mild mental retardation, and one with 37% score was sub trainable while 4 had moderate mental retardation.

Strabismus was found in thirteen (35.1%) subjects made up of esotropia in 7(54%) and exotropia in 6 (46%) subjects. Hirschberg and krimsky were ≤ 15 ^{0}/30 Prism Diopter (Table 4).

Refractive error was found in 32(86.5%) subjects. Hypermetropia in 4(12.5%), Myopia 7(21.9%) and

astigmatism 21(65.6%). Of the astigmatic subjects hypermetropic astigmatism was 14.3%, myopic astigmatism 14.3%, compound astigmatism 61.9% while simple astigmatism was 9.5%.

Table 4: Degree of squint in 13 subjects

Degree of squint	Number (%)
≤ 15^0/30PD	8(61.5)
≥15^0- 30PD	4(30.8)
>30-45PD	1(7.7)
Total	**13 (100.0)**

The myopia was ≤3D in 7 (70%),>3-5D in 1 (10%) and >5D in 2(20%). Hypermetropia was ≤ +2D in 12(66.7%) subjects, >+2D- +4D in 3(16.7%), and >+4D in 2(11.0%) subjects while Anisometropia of > +2D difference between the two eyes was found in 1(5.6%). Astigmatism was ≤1DCyl in 17 subjects and > 1DCyl in 4 subjects (Table 5).

Table 5: Refractive errors in 33 cerebral palsy patients

Astigmatism 21(65.5 %)		Myopia 7(21.9%)		Hypermetropia 4(12.5%)	
≤ +2DS	12(66.7%)	≤ +3DS	7(70%)	≤ 1DS	17
> +2DS -+4DS	3(16.7%)	> +3DS -5DS	1(10%)	> 1D	6
> +4DS	2(11.0%)	> 5DS	2(11.0%)		

Strabismus was found in 4 cases of Spastic Cerebral palsy, 1 in hypotonic, 1 mixed and 7 among the uncharacterized CP. Hypermetropia was more common in the spastic CP whereas myopia was more common with athetoid (hypotonic) CP, though this was not statistically significant.

The pupil reacted briskly in 30(81.1%) and sluggishly in 4(10.8%) subjects while 3(8.1%) were not reactive. Other findings were in one patient each Nystagmus, vernal conjunctivitis, and bilateral corneal scar with lower lid ectropion. Funduscopy revealed. Two (5.4%) subjects with tilted disc while 3 (8.1%) had optic atrophy and 1 had bilateral chorioretina scar.

There were significant socio economic issues noted during the study, as many parents had difficulties taking up care in form of correction of refractive errors and management of strabismus partly because of the need to pay out of pocket and fear of stigmatization

Discussion

There was a low response rate in this study, as shown by only 42.5% responding to our call to present for ocular assessment. This could be related to the psychological challenge faced by parents as a result of their children's health condition. This could also explain the wrong addresses given by some respondents

.

In this study a large proportion of the subjects were between 1 and 5years of age. This is comparable to other studies (Andersen, Irgens and Haagaas et al 2008:4-13; Sigurdardotttrir, Thorkelsson and Halidorsdottir et al 2009:356-63) and due to the fact that the signs and symptoms are not usually present at birth, they commonly evolve as the child is developing. The most common risk factor found in this study was birth asphyxia. Only 4(21.1%) of subjects had history of prematurity despite the fact that increased prevalence has been reported in premature infants (Burja, Seme- Ciglenecki and Gajsek- Marchetti 2004:39-43). Premature infants are vulnerable, in part because their organs are not fully developed, increasing the risk of hypoxic injury to the brain that may later manifest as CP. Other risk factors for the development of CP found in this study include meningitis/ encephalitis and hypoglycaemia as have been observed in previous studies (Odding, Roebweck and Stam 2006:183-91).

As reported by other workers (Beckung et al 2008: e187-92), Spastic cerebral palsy was more common than other types of cerebral palsy. Further classifications in quadriplegic, diplegic was not done because they were not classified by neurologists.

Ocular conditions found in these patients include refractive errors, strabismus, and optic atrophy. Majority (94.6%) of the patients had substandard visual acuity. This is in conformity with the reports of other researchers that there is a high rate of

vision /ocular defects among cerebral palsy patients (Lo Casio 1977:332-37; Govind and Lamba 1988:88-91), thus confirming that early eye screening is essential in these groups of patients for early detection, diagnosis and treatment.
Strabismus was diagnosed in thirty-five percent of subjects with exotropia being the most common type.

This is the experience of various authors though to a varying degree (Arnold 1955:852-856; Lo Casio 1977:332-37; Lagunju, and Oluleye 2007:71-5). The degree of squint was mainly in the range of 30 prism dioptre which is quite significant and will require early treatment. Many of the parents were however not keen about getting this corrected by surgery because they were more interested in finding a solution to the delayed developmental milestone of their children. A child with a squint is likely to have a poorer level of binocular vision especially depth perception. Tasks requiring fine depth discrimination will be more difficult.
About fifty percent of subjects had refractive error with astigmatism being the most common refractive error. This is unlike the findings of other authors (Govind et al 1988:88-91; Sasmal et al 2011:318-323) and may be because of the low sample size in this study. Significant Myopia was more common in the hypotonic CP. Of note is the fact that majority of these refractive errors are not high errors except in two of the myopic subjects. However, early detection and correction of these errors are important as the correction can make a significant difference in the children's ability to see and improved academic performance. Correction of the refractive errors will also help to maximize any residual vision in these children (Groenveld, Jan and Leader 1990:11-5) and can also help correct or reduce the amount of strabismus in those children with hypermetropia.
The finding of brisk papillary reaction in 80% of the subjects means the poor vision found in many of them was probably mainly due to cerebral visual impairment. Unfortunately, most of them did not do MRI /CT scan of the brain.
In this study only 8.3% of subjects have optic atrophy and this is similar to Govind et al (1988:88-91) who reported 10% prevalence of optic atrophy in their own series.
Other ocular conditions were uncommon, which confirms Lo Casio' (1977:332-37) report that ocular disorders are uncommon in these children, though a study with a larger sample size and probably a multicentre study will give better information.
The socio economic issues noted during the study, as many parents had difficulty taking up care in form of correction of refractive errors and management of strabismus is partly because of the need to pay out of pocket and challenges of stigmatization. This makes it important to involve counselors and financial support facilities for children who have cerebral palsy.
The strength of this study lies in the fact that it is the only study that looked at the degree of refractive error and strabismus in children with cerebral palsy.

We therefore conclude that Strabismus, refractive errors and CVI are the most common ocular abnormalities in children with Cerebral palsy. Also that early detection and early intervention is important to achieve best results. Therefore, multidisciplinary approach involving neurologists, ophthalmologists, counselors and physiotherapist should be embarked upon in managing these children from the time of diagnosis. Parental/care givers education cannot be overemphasized.

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Original Article

IMPROVING EDUCATION PROGRAMME AT PMTCT SITES IN LIBERIA: A KAP STUDY ON NUTRITION AND INFANT FEEDING AMONG CLIENTS OF 3 PMTCT SITES.

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ABSTRACT

This descriptive study aimed to determine gap in nutrition and infant feeding knowledge, attitude, and practice among the HIV positive mother under 6 months, in order to improve nutritional education program at PMTCT sites level. A total of 90 mothers of child under 6 months chosen at random were interviewed in three PMTCT sites. Data were analyzed by EPI INFO 3.5.3. The results showed in general fair knowledge, attitude but poor practice among the HIV positive mothers of child under 6 months in terms of nutrition and infant feeding.

Keywords: Nutrition, HIV/AIDS. Health Education, Health Promotion, Health System

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INTRODUCTION

HIV infection is a significant public health problem in Liberia. The prevalence stratified by gender shows a higher prevalence among the women. In addition Antenatal care surveys conducted in 2006, 2007, and 2008 show a prevalence of 5.7%, 5.4%, and 4.0% respectively among pregnant women (Ministry of Health and Social Welfare, 2006, 2007, 2008). Liberia also faces malnutrition due to the civil war which is exacerbated by poverty, food insecurity, poor dietary practices, low literacy levels and poor access to basic social services. The most vulnerable groups include people living with HIV (PLHIV).

Chronic malnutrition is endemic affecting 39% of the children under 5. In addition, 14% of women of child bearing age in rural areas and 7% in urban areas are undernourished. Also, 10% of Liberian women have a body index of less than 18.5 (United Nations of International Children's Emergency Fund & Ministry of Health and Social Welfare, 2010).

Addressing gaps in nutrition among PLHIV is essential because nutrition plays a vital role in the care and management of HIV and AIDS. However, very little data exist concerning these aspects of nutrition and infant feeding practices among women living with HIV in Liberia. Education programs are more likely to be effective if the characteristics of the target in terms of knowledge, attitude and practice are known.

This study consequently aimed to assess gap in nutrition and infant feeding knowledge, attitudes, practice of the mothers of child under 6 months, living with HIV so as to report on some effective ways to improve nutritional education program in these vulnerable groups.

MATERIAL AND METHODS

This cross-sectional study with descriptive aiming was conducted in November 2011 at the John Fitzgerald Kennedy Hospital of Monrovia, the Redemption Hospital and the Liberian Governmental Hospital of Bomi.

The study had recourse to both quantitative and qualitative methods. The sample was composed by 90 mothers of child under 6 months living with HIV, all of them chosen at random. The questions addressed to the mothers were related to the socio-economic characteristics of the household, nutrition, infant feeding option in the context of HIV/AIDS. To assess their diet, we chose two indicators, the number of daily eating occasion and the number of foods groups consumed through the 24 hours recall.

The data obtained were analyzed by EPI INFO 3.5.3. We used a chi-square test for independence with 95% level of confidence, to determine whether education level, occupation, are related to the diet and the choice of the infant feeding option.

RESULTS

Characteristics of the HIV mothers

Table 1: Characteristics of the Clients

Variables	Modalities	Frequency	Percentage
Occupation	Civil servant	4	4.40%
	Hairdresseur	8	8.90%
	Housewife	38	42.22%
	trader	28	31.10%
	Other	12	13.30%
	Total	90	100.00%
Education	Illiterate	32	35.60%
	Primary school	14	15.60%
	High school	30	33.30%
	College	14	15.60%
	University	0	0.00%
	Total	90	100.00%
Matrimonial status	Single	10	11.10%
	Co-habitation	42	46.70%
	Married	34	37.80%
	Divorced	0	0.00%
	Widow	4	4.40%
	Total	90	100.00%
Partner occupation	Civil servant	20	22,22%
	Farmer	8	8.90%
	Jobless	10	11.10%
	Trader	30	33.30%
	Taxidriver	6	6.70%
	Other	16	17.80%
	Total	90	100.00%

In general, most of the respondents were jobless (42.22%) with a high percentage of illiterate (35.60%). Moreover, most of their partners were traders (33.30%). Most of the clients lived in cohabitation (46.70%) or were married (37.80%).

Diet of the Clients

We found that 79.2% of the clients reported consumption of less than six food-groups and 81% had less than 3 daily eating occasions 24 hours before the interview; implying a poor or an inadequate dietary quality. Moreover the diet is not diversified. The nutritional practices were not influenced by the level of education (p=0,256), and occupation (p=0,09)

Infant feeding option in the context of HIV

Out of the 90 interviewed 30 (33.33%) has chosen to breastfeed while 60 (66.67%) have given replacement food. The choice of the infant feeding option was not related to the mother level of education (p=0,233) and occupation (p=0,015). The reasons for choosing breastfeeding or replacement food are listed below.

Table 2: Reasons for choosing exclusive breastfeeding or replacement food

	Reasons	Frequencies	%
Exclusive Breastfeeding options	Stigmatization	9	29.0
	No financial support	7	24.0
	The clinician advised me	8	26.0
	Maternal milk is good for baby	6	21.0
	Total	30	100.0
Replacement food	To protect my baby fron HIV	34	56.0
	The clinician advised me	26	44.0
	Total	60	100.0

Stigmatization remains the main reason which brings the women to choose to breastfeed their baby. Even though 21% of the mothers answered that the maternal milk is good for the baby, none of them were able to cite at least one component of the maternal milk.

When the mothers of child under 6 months were asked about what they have to do to protect their baby from HIV during exclusive breastfeeding: 80.4% answered that they take their medicine while 9.6% do not know what to do.

For those who breastfeed the child, they know the recommendation and say that they want to avoid mix feeding but they don't know that the breast milk already contains water

When asked about the reason of choosing the replacement feeding, 56% of the targets interviewed answered that they want to protect they baby against HIV, 44% that they have been advised by the clinician.

Respect of AFASS (affordable, feasible, acceptable, sustainable, safe) Criteria when Giving Replacement Food.

The assessment of the actions taken by the mothers to ensure the safety of the replacement food revealed that only 2% used mineral water and when they could not afford it 33% boil the water. None of them used cup feeding.

Disadvantages/advantages of breastfeeding and the replacement food according to the mothers

Only 68% mothers who have chosen to breastfeed knew the main disadvantages of exclusive breastfeeding which is the high the risk of HIV transmission.

Out of the clients who have chosen the replacement feeding, only 16% answered the high risk of diarrhea and other infection. None of the women interviewed, mentioned as disadvantage the risk of malnutrition.

DISCUSSION

The study reported on fair knowledge and attitude but poor practice of the positive HIV positive

mothers, toward nutrition and infant feeding education.

Indeed, a high proportion of the mothers (79.2%) reported consumption of less than six food-groups, implying a poor or an inadequate dietary quality as option, which revealed a lack of nutritional defined by Swindale and Ohri-Vachaspati (2004). In average they have 2 meals per day which is not enough when the energetic needs are important[5]. We found that the nutritional practices were not influenced by the level of education (p=0,256), and occupation (p=0,09), contrary to Tang AM (2003) who highlighted that practices are influenced by social, cultural, economic factors as demonstrated and also by the health workers.

Table 3: Avantages/Disadvantages of Breastfeeding and the Replacement Feeding According to the Mothers

	Breastfeeding option (n=30)	%	Replacement Food (n=60)	%
Advantages	The milk is good for the baby	21.0	Protect the baby against HIV	95.0
	You don't spend money to buy milk	79.0	Other answers	5.0
Disadvantages	The risk of HIV transmission	68.0	High risk of diarrhea and other infections	16.0
	Other answers	25.0	Stigmatization	25.0
	Do not know	7.0	Do not know	59.0

Other studies have shown that low socioeconomic status, level of education, personal beliefs, availability of food, and low nutrition knowledge as contributory factors to poor dietary practices. The poor dietary practices among the PLHIV may also result from loss of appetite and anorexia, thus reducing the frequency of meal and variety at the very time when their requirements are higher (Walsh, Dann Hauser, & Joubert, 2007; Kim, Spiegelman, Rimm, & Gorbach, 2001).

Infant feeding recommendations for HIV mothers must take into account the local environment and conditions to provide the most accurate information on risks and benefits of breast-feeding and replacement feeding. In the absence of these data, physicians and other hospital staff are faced with deciphering information from other countries and settings, which face conditions different from those of their populations (Suryavanshi et al., 2006).

Exclusive breastfeeding is recommended because it protects infants from morbidity and mortality whether or not HIV related (Victora et al., 1987) . In addition, Coutsoudis and al (1999) reported that HIV-exposed infants who were breastfed exclusively for at least 3 months had a lower risk of HIV infection than mixed-fed infants.

Concerning our study, most of the mothers interviewed (66.67%) have chosen the replacement feeding but few of them are stingy to the AFASS criteria. Indeed, none of the women interviewed mentioned as disadvantage the risk of malnutrition for the child, if the food is too diluted. One problem met by the women who have chosen the replacement feeding is the sustainability of this. Even though they receive at the facility 2 cans of milk every two weeks free of charge, they are sometimes unable to buy the cans in case of stock out before the supply. Therefore, they manage the cans and diminish the number of milk spoon recommended.

For those who have chosen to breastfeed, it is mainly because of stigmatization. However, there is a lack of awareness among these women on the importance and benefits of early initiation, the respect of good positioning during breastfeeding to avoid many problems such as insufficient breast milk and sores breasts (Kelleher, 2006).

The choice of the infant feeding option was not related to the mother level of education (p=0,233) and occupation (p=0,015).

CONCLUSION

This study highlighted the need of improving education program at PMTCT site in Liberia.

Besides, it will be interesting to deepen researches in order to elaborate a practical guide of nutrition for people living with HIV AIDS in Liberia.

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Original Article

Traumatic Brain Injury and Metabolic Dysfunction Among Head Injured Patients in a Tertiary Hospital in North-Central Nigeria

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Abstract

Traumatic Brain Injury (TBI) is a common health problem which is one of the main causes of chronic disability and it is associated with hormonal and metabolic disorders. This work was carried out to investigate the relationship between some stress hormones (i.e. prolactin and cortisol) and plasma glucose level in TBI patients. Twenty-five TBI patients were included in the study consecutively. All patients underwent basal hormonal and plasma glucose evaluation within 24 hours of admission. One of the patients died during the acute phase. The results of the study show that prolactin and glucose levels were positively correlated with the Glasgow Coma Scale (GSC). Cortisol levels were observed to be highest in the moderate TBI group with GSC score of 9-13. The results also showed that 80% (20 patients) of the patients were age 40 years and below and 84% (21 patients) were males. In conclusion, present data show that prolactin, cortisol and plasma glucose are disturbed in TBI. The disturbances in the levels of prolactin and glucose are related to the severity of TBI. However, there is no direct relationship between cortisol and severity of TBI. Our results also show that TBI is more common in males and young people.

Keywords: Traumatic Brain Injury, Plasma Glucose, Cortisol, Prolactin

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Introduction

Traumatic Brain Injury (TBI) is a common health problem and is one of the main causes of chronic disability and death among young adults through a variety of mechanisms, and is now recognised as a cause of neuroendocrine dysfunction (Mazaux and Richer, 1998). TBI-induced neuroendocrine dysfunction was previously thought to be a rare occurrence; however, it is now being increasingly diagnosed (Ghigo *et al*., 2005). Recent studies of traumatic head injury patients in the chronic recovery phase from their injuries have indicated specific deficits in their general health, vitality, and mental health with depression and anxiety being particularly common (Colantonio *et al*., 1998; Morton and Wehman, 1995). These psychosocial consequences often become the major impediment to successful rehabilitation (Morton and Wehman, 1995).

However, assessment of pituitary functions in patients with TBI is not a routine procedure in endocrine practice. Hypopituitarism due to TBI may be partial or complete, and 20-50% of patients who were tested several months and years after head trauma have been demonstrated to have some degrees of pituitary dysfunction (Leal-Cerro *et al*., 2005; Tanriverdi *et al*., 2006).

Mortality and morbidity rates are significantly lower in patients treated in a postoperative surgical intensive care unit (ICU) with intensive insulin therapy, titrated to maintain strict blood glucose control (blood glucose levels at 80–110 mg/dl or 4.44–6.11 mmol/l), than in patients receiving conventional therapy (Van den Berghe *et al*., 2005). Strict glycaemic control with low target ranges invariably carries a risk of inadvertent hypoglycemic episodes. One study has documented an association between incidental hypoglycemia and an increase in short-term complications or mortality (Krinsley and Grover, 2007). Several studies have nevertheless reported a potentially higher incidence of hypoglycemia in patients under strict glycaemic control (Thomas *et al*., 2007).

There has not been any study to the best of our knowledge conducted in the North-Central Region of Nigeria to investigate the relationship between stress hormones and plasma glucose in the early phase of TBI. Therefore, we set out to study the stress hormones (cortisol and prolactin) and plasma glucose in the early phase (within 24 hours of trauma) in 25 patients with TBI. Additionally, the relationships between the serum level of the stress hormones and plasma glucose, and severity of the TBI were investigated.

Patients and Method

Patients

Twenty-five patients with TBI (16 men, 9 women; age 42±13.4, range 17-84 years) who were admitted via Accident and Emergency (A&E) Unit and managed by the Neurosurgery Unit of the University of Ilorin Teaching Hospital were included in the study consecutively between January and April, 2013. The study was approved by the Ethical Review Committee of the University of Ilorin Teaching Hospital. The instrument used included semi-structured questionnaire for the clinical details and the laboratory assessments.

The demographic details and time of presentations were noted in the information retrieved. The level of consciousness of the patients was assessed by the same investigators according to Glasgow Coma Scale (GCS) as soon as the patients were admitted to A&E Unit. The post resuscitation GCS score was used to classify TBI into; mild (GCS 14-15), moderate (GCS 9-13) and severe (GCS ≤ 8) (Mena *et al*., 2011). None of the patients had a history of any known pituitary disorder and all the patients were off any drug affecting hypothalamo-pituitary function.

The cause of TBI was road traffic accidents in all the patients: 9 (36%) was due to motorcycle accident and 16 (64%) was due to motor vehicular accident

Basal Hormonal Assay in the Acute Phase

All patients underwent basal hormonal evaluation within the first 24 hours of admission to the A&E. blood samples were taken between 8.00-9.00hr. No patient received glucocorticoids, dopamine, ketoconazole or calcium channel blockers before blood specimen collection. Basal hormonal levels of prolactin and cortisol, and random blood glucose were measured. Menstrual history was obtained from women or their relatives.

Hyperprolactinaemia was defined when basal level is higher than the upper normal level of the reference limit (male: 18ng/ml, premenopausal women: 29ng/ml, postmenopausal women: 20ng/ml). Normal range of cortisol is (Adults: Morning: 5-23µg/dl, Afternoon: 3-13 µg/dl; Children: Morning: 3-21µg/dl, Afternoon: 3-10 µg/dl). Ref limits for glucose: Postprandial of up to 10.0mmol/l.

Analytical Method

All serum hormones were measured by using ELISA

method following the instructions on the commercial kits. Cortisol and prolactin were measured by using AccuBind ELISA kit, (Monobind Incorporated, Lake Forest, CA USA). Glucose was estimated using Accucheck (Rhode Diagnostics, Indianapolis, IN, USA).

Age Range (years)	Number of Patients
10-25	13
26-40	7
41 and above	5

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for windows. All information retrieved was entered into SPSS version 20.0 and manual corrections for errors were done and data were subjected to Duncan post-hoc test. Data were presented as mean±SD, normally distributed values between two variables were compared by unpaired t-test. More than two variables were compared by using one-way ANOVA test and Duncan's test was performed for post-hoc analysis.

Results

Twelve (48%) patients had mild (mean GCS: 14.4±0.8), 8 (32%) had moderate (mean GCS: 11.1±1.4) and 5 (20%) had severe (mean GCS: 6.1±1.1) TBI. One of the patients, a male, who had severe TBI died within 72 hours of admission. Our results showed that 21 of the patients representing 84% were males and only 4 were females representing 16% of the patients. The age range of the patients in Table 1 shows that 13 (52%) were within the age range of 13-25 years, while 7 (28%) of the patients were within the range of 26-40 years and 5 (20%) were older than 41 years.

Table 1. The age range of the patients

The results of the serum hormones and glucose in Fig. 1, showed that serum prolactin and glucose were significantly ($p<0.05$) higher in the TBI groups compared to the control group. However, serum cortisol was only significantly ($p<0.05$) higher in the moderate TBI group than the control.

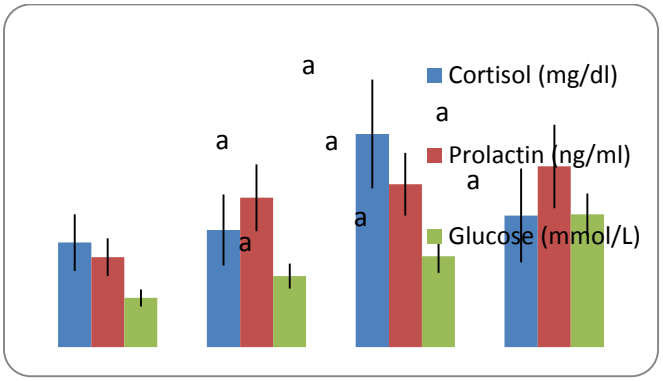

Fig. 1. Changes in Plasma cortisol, prolactin and glucose in the early acute phase of TBI. Data are presented as mean±SD, a indicates $p<0.05$ vs control for the parameters.

Discussion

The results of this study showed that TBI is overwhelmingly a male gender problem with about ratio 5 to 1. This is consistent with earlier reports which demonstrated a ratio of 3:1 (Greenwal *et al.*, 2003). However, our ratio of 5:1, may not be unconnected with the fact that, in our environment, motor cycle is used as a form of public transport and it is an exclusively a male business. The results also demonstrated that more than half of the patients with TBI were in the age range of 10-25 years. It has been reported previously that the highest injury occurs in young men between that age of 15 and 24 years (Murdoch and Theodoros, 2001). Some of the reasons adduced for the preponderance of TBI in young adult is because overall, motor vehicle or related transportation accidents and falls comprise the most common cause of TBI. Transportation accidents, particularly for young males (15 to 24 years), by some estimates account for more than 50% of all head injuries (Murdoch and Theodoros, 2001).

We demonstrated that there were changes in basal stress hormone levels and plasma glucose following traumatic brain injury (TBI). Our research also showed that plasma glucose and stress hormones changes in TBI are related to the severity of the TBI.

The pituitary gland responds to acute traumatic events and several changes in the circulating hormone levels become apparent during the first hours or days after injury, and may continue for the period of acute critical illness (Woolf, 1992). Elevated serum cortisol level during the initial phase of trauma has been previously reported in patients with mild and moderate TBI (Barton *et al.*, 1987). Additionally, a positive correlation between the severity of the injury and cortisol

concentration has been shown in patients with mild or moderate TBI, but not in those with severe injury (Feibel *et al*., 1983). In contrast, primary or secondary adrenal failure has been shown in 15% of patients with moderate to severe injury, 7-60 days after TBI (Dimipoulou *et al*., 2004). In the present study, cortisol levels were positively correlated with mild and moderate injury, whereas cortisol level was negatively correlated with severe TBI. The result of our study is different from that of Tanriverdi *et al*., 2007, which showed negative correlation between cortisol levels and severity of TBI.

Severe injury may give rise to more hypothalamic or pituitary damage, and results in blunted hypothalamic-pituitary axis response to stress (Tanriverdi *et al*., 2007). Also, other factors involve in the control of cortisol secretion may come into play during severe TBI.

In acute phase of TBI hyperprolactinaemia has been reported in more than 50% of the patients who had moderate or severe injury (Bondanelli *et al*., 2005). A positive correlation between severity of trauma and the prolactin levels has been demonstrated in previous studies (Agha *et al*., 2004; Bondanelli *et al*., 2005). In this study prolactin is higher in all the TBI groups than in the control group and this is related to the severity of the injury. Stalk lesions or hypothalamic lesions are generally related to hyperprolactinaemia. Thus the normal prolactin in all our patients was suggestive of pituitary lesion. Therefore, it is tempting to speculate that mild injuries may generally affect pituitary but severe injuries may affect the stalk and hypothalamus. Our result is similar to the findings of Tanriverdi *et al*., 2007, which showed positive correlation between serum prolactin and severe TBI.

Hyperglycaemia is frequent during acute brain injury such as ischaemic stroke, intracranial haemorrhage or traumatic brain injury and is associated with increased morbidity and mortality (Bhalla *et al*., 2003; Bilotta *et al*., 2007). TBI also leads to a profound increase in glucose utilization (hyperglycolysis) that can persist for up to one week and alter the ability to use ketone bodies as energetic substrates (Robertson *et al*., 1991). Our study shows a positive correlation between plasma glucose and severity of TBI. Hyperglycaemia in the trauma patients, as in other critically ill patients, is caused by a hypermetabolic response to stress (Eakins, 2009). Even though cortisol is one of the major stress hormones, our study does not show direct relationship between plasma glucose and cortisol. This may be due to the disruption of hypothalamic-pituitary axis leading to reduced production of corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) which is essential for the production of cortisol. Severe trauma may give rise to more hypothalamic or pituitary damage, and results in blunted HPA-axis response to stress[27]. In the severe TBI group, our study shows reduced cortisol concentration. The high blood glucose in the severe TBI group could therefore be due to other stress hormones, such as prolactin which shows direct relationship between the severity of TBI and plasma glucose. Holbein and colleagues pointed out in their study that hyperglycaemia following severe TBI down regulates GLUT 1 transporter in the blood-brain barrier thereby decreasing endothelial flux of glucose even at high arterial blood glucose levels, thereby leading to reduced cerebral glucose availability despite an adequate arterial supply (Holbein et al., 2009). This would increase lactate production, decrease intracellular pH and cause cellular distress, which results in impaired metabolic activity and possibly adverse outcome (Vespa et al., 2006; Holbein et al., 2009).

In conclusion, our data clearly demonstrate that TBI is associated with hyperglycaemia and disturbed stress hormones such as prolactin and cortisol. The study also shows the need to institute intensive management of plasma glucose following TBI so as to improve outcome.

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Original Article

Patterns of Reproductive Health and Sexual Behaviours Among Female Undergraduates in Osun State South West Nigeria.

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ABSTRACT

Background: Sexually transmitted infections including HIV/AIDS in addition to induced abortion continue to exert huge cost in human lives and material resources especially in developing countries. Among the morbidities resulting from these reproductive health diseases are chronic pelvic pains and infertility with further grave consequences on marital harmony and quality of live that may last the entire life of the individual woman.
Young Females in the age group of those in Colleges and Universities constitute majority of the victims.
Objectives: To determine the practices and the risks of unsafe reproductive health behaviors among female undergraduates in south western Nigeria.
Methodology: A cross-sectional survey of reproductive health knowledge, sexual practices as relates to contraceptive use, multiple sexual partners, unwanted pregnancy and induced abortion among the females students in three Universities in Osogbo, Osun State, South Western Nigeria. The responses were analyzed using descriptive statistics, and bivariate and multivariate analysis.
Results: Many Nigerian undergraduates initiated sex at early age and have multiple sexual partners. These two factors are statistically significantly associated with risk of induced abortion ($p < 0.05$). Mothers did not give safe reproductive health information to their daughters. Many respondents were aware of STI and modern contraception, yet emergency contraceptives were being used inappropriately by Nigerian Undergraduates.
Conclusions: There is high magnitude of unsafe reproductive health behaviours and sexual practices among undergraduates in this environment. This calls for behavioral change intervention in other to enhance safety in reproductive health lives of the female undergraduates in south western Nigeria.

Key words: Reproductive health, patterns, undergraduate, Behaviors safety.

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INTRODUCTION

Adolescents who pursue higher education are much likely to delay marriage and childbearing. The age of sexual maturity is declining and young females are starting sexual activities at earlier ages than their parents. (Salako &Sofowora,2002; Federal Office of Statistics Lagos 1990). Premarital sex also appears to be on the increase as a result of the combination of the reasons mentioned above (Umolysho et al 2004; Araoye &Adegoke, 1996).

Sex before marriage, mostly unsafe, exposes the adolescents to risks of Sexually Transmitted Infections (STI) including HIV/AIDS and unwanted pregnancy, the later often result in the procurement of clandestine abortions with its attendant dangers, (Otiode, 2001; Adeleke et al 2006. Markiuwa – Adebusoye, et al 1997).

Effective and appropriate contraceptive methods use prevent unwanted pregnancy. (Marston C, Cleland J. 2003, Haggaid Emergency Contraception. 2003) and STI. However, prevalence of contraception is low in Nigeria especially among the unmarried young females. (Salako, 2006). There is also regional variation in the country, with the south west having highest and the North West the lowest contraceptive use prevalence. (Goliber et al 2001).

The magnitude of unmet need for contraception is high, it is estimated at between 24 - 26% in developing countries including Nigeria. (National Population Commission 2009; Okeh, et al 2011) Sexually active adolescents both in school and out of school constitute a significant component of the unmet need for contraception and unsafe reproductive health issues.

Factors contributing to low contraceptive use include negative religious attitude, illiteracy, high cost and male disapproval. (Tokeh, et al 2011) Added to these is the unfriendly attitude of the family planning service providers to the adolescents and young adults such as University undergraduates who are often not married.

Youth age is characterized by tendency for risky behaviors such as night parties, alcoholism and drug abuse as well as negative peer influence. These behaviors predispose to casual and unsafe sex. Against the background of active sexual activities and poor contraceptive access and use among these young adults in higher institutions, this study sought to explore the situation among the female undergraduates in this environment.

METHODOLOGY

Study Area: Osun State is in South Western Nigeria with a population of about 3.5 million. (National Population Commission 2009) There are three tertiary institutions in the state capital namely Osun State University, LAUTECH College of Health Sciences and Fountain University. Osun State University has only the main campus (with two Colleges) in Osogbo. LAUTECH has only the College of Health Sciences in Osogbo city. Fountain University is a private University unlike the other two that are government-owned and has only one campus located in Osogbo. The total population of these institutions is about fifteen thousand students.

Study design: This study was a descriptive cross-sectional study of patterns of sexual behaviors and associated risks among female University undergraduates in South Western Nigeria. Registered female students of these Universities with age 15 years and above constitute the target population.

Sample size estimation: Using the Leslie Fischer's formula for the calculation of sample size for a population more than 10,000, a sample size of 400 was calculated. This was increased to 500 to take care of possible non-response from this study.

Sampling method: A multi-stage sampling method was used in selecting respondents into this study. In the first stage, two out of three Universities were selected by simple random sampling technique employing simple balloting. Osun state University and Fountain University were selected. In the second stage, a list of all Colleges and Departments in each University was obtained from the University authorities. Using simple random sampling, two of three Colleges, and two Departments per College were selected in Osun State University employing simple balloting. Similarly, four of six Colleges and two Departments per College were selected from Fountain University. Questionnaires were proportionately allocated to the twelve departments selected.

In stage three, stratified sampling method was used in selecting the classes using their level of education as the stratifying factor. One level or class per department was randomly selected. In

stage four, Respondents were recruited into the study using a systematic sampling of one in three according to the day's sitting arrangement in the lecture hall. Questionnaires allocated to the classes

were distributed until they got exhausted. In the class where questionnaires were not exhausted, another class was chosen using simple random sampling and respondents were recruited in the same way.

Research instruments: A semi-structured self-administered questionnaire was used. This was pre-tested among University students in Ile Ife in Osun State. Study was carried out during the Harmattan semester of 2013/14 academic session. The variables sought include socio-demographic data, sexual practices (such as age of first coitus, number of sexual partners) and pattern of condom use, awareness of sexually transmitted infections (STI) and sources of their knowledge as well as Voluntary Counseling and Testing HCT were assessed.

Ethical clearance**:** Ethical clearance was obtained from UNIOSUN College of Health Sciences research ethical review committee. Permission was sought from the selected Universities' and departments' head. . Furthermore, informed consent was obtained from each respondent. Respondents were assured of confidentiality of findings.

Data management: The Statistical Package for Social Sciences (SPSS) software version 17.0 was used for data entry and analysis after sorting out the questionnaires. Validity of data was done by double entry and random checks for errors and outlier values. Relevant frequency distributions tables and summary indices were generated. The Chi-square test was used to demonstrate relationships between categorical variables. Regression models were used for multivariate analysis of quantitative variables while level of statistical significance was set at P-values ≤0.05 in determination of relationships between categorical variables.

RESULTS

Table 1.0 shows the socio-demographic features of respondents; a total of 494 respondents distributed across the four levels of study returned completely filled questionnaires giving a response rate of 98.8%. The highest number of respondents was from the 200 level. The age group 20 to 24 years was the most common, less than 2% were married and Christianity was the dominant religion among the respondents.

Table 1.0 shows the socio-demographic features of respondents; a total of 494 respondents.

Table 2.0 shows the awareness and knowledge of certain aspect of safe reproductive practices. Among 390(78.9%) who had family life education (FLE) or Reproductive Health Education.

Table 1: Sociodemographic Characteristics of Respondents

Socio-demographic (n=494)	**No**	**%**
Age		
15-19	193	39.1
20-24	238	48.2
25-29	54	10.9
30 and above	9	1.8
Class/level		
100	47	9.6
200	214	43.3
300	162	32.8
400	71	14.4
Marital status		
Married	8	1.6
Single	396	98.4
Religion		
Christian	368	74.5
Muslim	105	21.3
Traditional	14	2.8
Others	7	1.4
All females, all students		

Table 2.0: Safe Reproductive Health Knowledge of the Undergraduates.

PARAMETERS	POSSITIVE (%)	NEGATIVE (%)
Knows own HIV status.	143 (29)	351(79)
Knows partners HIV status.	49 (10)	445 (90)
Safe period	217(44)	277(57)
Knows about STI	356(72)	138 (28)
Informed about modern Contraceptives	346(70)	148(30)
Know Ovulation signs	207 (42)	287(58)
Aware of Own Ovulation	198(40)	296 (60)
Other RHI	346(70)	148(30)

The sources of their awareness was depicted in Figure 1.0 and included the following; course 140(28.3%), topic of discussion 271(54.9%) on school's assembly ground 108(21.9%) and at a seminar 294(59.5%). Other sources are internet, health workers and friends.

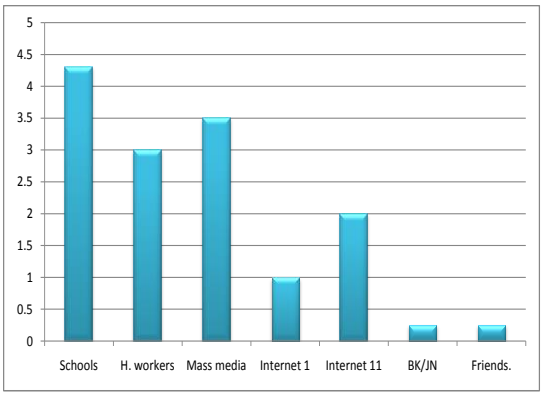

Figure 1.0: Sources of Reproductive Health Information to the Nigerian Undergraduates.

Ten percent of respondents knew own HIV status while 29.0% were aware of partners HIV status. Most respondents (72.0%) knew about group of diseases referred to as sexually transmitted infections (STI) such as HIV, gonorrhea and syphilis, while 23(4.6%) had been treated for STI.

Forty-four percent and 42% of respondents had knowledge of safe period in a menstrual calendar and self-ovulation signs respectively while 70% were awareness of modern contraceptive methods and how they are used. Sources of information to the respondents on reproductive health matters are shown in figure 1.0. Mass media including Television, radio and print media were the main sources in 80.0%. Health workers in 18.0%, internet (i) and (ii) (in this study internet i refers to standard web sites including Wikipedia while internet ii refers to social interactive sites such as face book, twitter and Twoo webs) were responsible in 25.0% while friends and books accounted for another 15.0%. Parents especially mother was the source in 2.0%. There was multiple reporting of sources.

Early age of initiating first sexual intercourse and multiple sexual partners were found in 50.0% and 30.0 % of the sexually active respondents respectively. Non-use of contraception including barrier methods was found in 50.0% while EC was used more frequently. Eight and 7.0% respectively had experienced unwanted pregnancy and undergone induced pregnancy termination; of the later about one third had repeated induced abortions. Thirty percent of the sexually active had multiple sexual consults and about half knew their male partners had other sexual partners. Oral and anal sex was practiced by 20.0% and 19.0% respectively. Casual sex often at night parties and use of alcohol was reported by 7.0% and 12.0% respectively (Table 3).

Correlations of sexual behaviors of the respondents using bivariate analysis was done. There was significant association between awareness of STI and number of sexual partners ($p=0.005$), but awareness of STI was not statistically significantly associated with pregnancy or ever-use of contraceptive ($p>0.05$). A statistically significant association existed between having procured abortion and ever used contraceptive ($p<0.05$), but no statistically significant relationship was found between having procured abortion and having multiple sexual partners ($0>0.05$). Similarly, there was significant association between age at first sex and number of sexual partners ($p<0.05$) (Table 4.0).

Table 3.0: Sexual Behaviors of Undergraduates

BEHAVIORS	Positive	%
Sexually Active n=(494)	186	37.7
Early Age of First Coitus (n=186)	93	50
First Coitus not Planned (n=186)	116	62.4
Multiple Sexual partners	56	30
Partners have other Consults	35	18.8
Nonuse of contraception	93	50
Had unwanted pregnancy	15	8
Terminated Pregnancy	13	7
Treated for STI	9	5
Sex at party	13	7
Casual Sex	9	5
Practice Oral sex	37	20
Practice Anal sex	19	10

In Table 5.0 multivariate analysis revealed that respondents who were aware about STI were 3.9 times more likely to have single sexual partners compared to those who were not aware. . Respondents who have had abortion have no higher likelihood of having more sexual partners compared to those who never had abortion. Respondents whose age at first coitus is above 15 years were three times more likely to have single sexual partner and twice less likely to have experienced unwanted pregnancy compared to those whose sexual debut was at a younger age.

	Singe Partners		Ever been pregnant		Never used male condoms	
	OR	95% CI, P	OR	95% CI, P	OR	95% CI, P
Heard about STI (Constant = no	3.92	1.53-10.06 0.003	1.20	0.48-2.99 0.358	1.12	0.60-2.08 0.365
Had abortion (Constant = no	1.15	0.43-3.60 0.351	3.70	7.56-18.8 0.001	8.50	3.29-22.0 0.001
Age at first sex (Constant = <15	0.03	5.4-14.7 0.001	0.55	0.62-4.89 0.329	0.64	0.10-4.08 0.3262

DISCUSSION:

Reproductive health education is important for safe sexual health behaviour and practices for every adult particularly the young adults as most undergraduates who are beginning sexual relationship. Majority of the respondents have had pieces of information about RH in the form of family life education and educational institution is the main source. The parents particularly the mother who is expected to be the source of early

information on safe reproductive health to their daughters were lacking in this responsibility, this is in agreement with the report by Asekun-

Olarinmoye et all in a study titled practice and context of sex education within a family setting: A study among Adolescents in a rural community in Osun State, South west, Nigeria. (Asekun-Olarinmoye, et al 2011).

A large percentage of respondents were aware of sexually transmitted infections especially Gonorrhea, Syphilis and HIV, while about 4% had been treated for same in the past, this is higher when compared to a 1.5% previous treatment for STI in a recent study amongst graduate students in China. (Zhou, et al 2013). Awareness of STI appears to exact a checking effect on having multiple sexual partners and this relationship was found to be is statistically significant in this study, this shows the information is properly utilized.

Only about ten percent of respondents knew their HIV status, however, three times that number knew the status of their male partners. This suggests that many the respondents demanded HIV status of their sexual partners even without knowing their own status. However, about fifty percent expressed desires to have HIV Counseling and Testing (HCT). This later finding is encouraging as it demonstrates a new positive attitude to safer sex.

Many respondents were sexually active and engaged in risky sexual practices such as, early age of first sex (which is defined as first coitus before the age of fifteen years in this study). This was found to be significantly associated with having multiple partners and unwanted pregnancy. Casual sex and sex at night party were also practiced by many respondents. These types of unsafe sex behaviors are common in this age group. **(**Hoque and Ghua,. 2012; WHO 2007; Qiaoqin, et al, 2006)

These behaviors call for intervention designed to effect positive behavioral change as shown by awareness of STI in this study.

Awareness of modern contraceptives was high, ever use and use of contraceptives at the last sexual act was reported among less than fifty percent of respondents. This is similar to reports by Rapossi and Arane (1994) in Spain in their studies on University students. This finding reveal the magnitude of unmet need for contraception in this age groups and calls for program of intervention to reduce the problem of unsafe sexual practices.

In figure 1.0, the main sources of information on safe reproductive health including contraceptives are the educational institutions programmes. Others are mass media, health workers, friends, journals and internet especially through the social media. This is contrary to the finding of Adeleke and Olowookere in the study on anaemia in the booked pregnant women in Osogbo where mass media was the dominant source of health information. (Adeleke & Olowookere, 2010). However, this may be a reflection of differences in the population studied. We recommend that information obtained from the social media must be treated with caution. The finding that more than a third of the sexually active demanded their partners use condom at the last coitus is encouraging as it is a reflection of women being in control of own sexual practices to ensure safety.

Knowledge about signs of ovulation including awareness of own ovulation (one of the natural family planning methods) is found in 2 out of 5 respondents, this is below average particularly when it is viewed from their educational status. This may be among the reasons the use of emergency contraception (EC) was high, probably most of the uses of EC have no valid indications, as many studies had reported poor level of Knowledge and use of EC. (Nworah, 2010, Arowojolu & Adekunle 2000; Bello et al, 2009; Wong 2007).

About ten percent of the respondents had unwanted pregnancy, most of which were terminated by induced abortion. Similar findings were reported by previous studies. (Wong 2007, Oriji, et al 2009, Bankole, et al., 2006). This is another reflection of unmet contraceptive needs and it is rather incongruent with the high level of awareness of contraception found in the study. Curiously, those who had procured abortion were using contraceptives and did not have higher number of sexual partners when compared to those who never had abortion. Possible explanation may be that the contraceptives being used were not appropriate and were not effective. The finding that emergency contraception was used frequently in this study corroborates this position.

Conclusion

This study showed poor knowledge of safe reproductive health and occurrence of unsafe reproductive health practices among female undergraduates in this environment. There is a need for behavioral change interventions targeted at the pre-university age group (less than 15 years) and mothers, to address sexual practices, education about contraception and voluntary counseling and testing HCT. These measures if implemented will promote safety in reproductive health matters among Nigerian female undergraduates.

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Author Contribution:

N.A.: Participated in all the stages of the study from conception, design, production and administration of data instruments, analysis and writing of the manuscript.

E.O.: Was involved in the design, production and administration of data instrument. He also contributed to results and writing the manuscript.

W.O.: Participated in the design, production and administration of data instrument, statistics and writing of the manuscript.

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Original Article

Patients' Perception of a Symptomatic Tinnitus among Nigerians: A multi-institutional study.

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Abstract

Tinnitus is a very common otologic symptom presented by patients worldwide yet it's a poorly understood disorder. This study is aimed at assessing the perception of patients of their tinnitus.
A multi-center prospective study carried out in Ear, Nose and Throat Department of two tertiary health institutions in Nigeria over a period of 12 months using a semi-structured Modified Tinnitus Impairment Questionnaire (THI-12) from Greimel et al after an informed consent. Information collected is entered into SPSS statistical software version 16.0 and analysed descriptively and results presented in tables and figures
All consecutive adult patients who presented for the first time at these clinics with complaints of tinnitus were included in the study.
A total of 168 patients with tinnitus consented to partake in the study comprising 38.2% males and 61.8% females with M: F ratio of 1:1.6. The age ranged from 18-80years, with a mean of 46.8 years. About 48.5% had tertiary education and 38.1% are self-employed, duration varied from 3 days to 25 years. The degree of discomfort experienced in the presence of tinnitus was said to be much in 16.2%, little in 63.2% while 17.7% experience no discomfort and no response in 2.9% patients. The degree of hearing loss varied with severity of tinnitus however none had profound hearing loss.
Tinnitus is still a problem among the sufferers as 16.2% describes it as bothersome, the need for proper evaluation of all medical problems and the need for a community based programme to assess the degree of disability of clinical tinnitus in the population will go a long way in defining its gravity.

Keywords: Tinnitus; hearing loss; Perception; Impairment

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Introduction

Tinnitus is derived from the latin word "tinnire" which means ringing in the ear and this was first introduced by Pliny and Elder in AD 23-79 (Feldmann, 1991). Tinnitus is a very common symptom presented by patients worldwide yet it's a poorly understood disorder. The phenomenon is as old as existence of mankind and as such, different meanings have been given to it. In a book titled "Model of Tinnitus" It was defined as a sound perceived in the ear for more than five minutes in the absence of any external acoustical or electrical stimulation of the ear and not occurring immediately after exposure to loud noise (Stephens, 2000) previous writers have described it as a phantom auditory perception (Jastreboff, 1990) while some described it as a head noise (Fowler, 1965). Tinnitus itself is a symptom and not a diagnosis. It is characterized as either objective or subjective depending on the ability of the examiner to perceive the tinnitus. Tinnitus differs from the word auditory hallucination which is usually described as a form of repetitive voices or musical themes and is common among the elderly, psychiatry patient or patients suffering from chronic alcoholism (Borka & Linda, 2008).
Data from the National Center for Health Statistics show that tinnitus is more common in men than women and increases in prevalence with advancing age. It is frequently associated with hearing impairment (Adams et al 1999). Previous studies have shown a tinnitus prevalence of 38% in patients less than 40years and 62% in those who were 40years and above (Coles,1984). Studies exploring the relationship between melanin pigmentation in humans and tinnitus shows that tinnitus is commoner among the blacks than the Caucasians (Borka & Linda, 2008). Study of US patients with chronic tinnitus show that only 25% of sufferers perceived their tinnitus as bothersome (Seidman & Jacobson, 1996) while only 14% of sufferers in the United Kingdom considered their tinnitus a significant problem (Seidman & Jacobson, 1996). Perception of Individuals differ in the manner they perceive a particular stimulus or situation and this may have led House to stating that "The same level of tinnitus may be described by one patient as intolerable, may be describe by another as barely noticeable (Seidman & Jacobson, 1996). The factors responsible for this difference in perception is not well understood and this have made the evidence based therapy unsuccessful.

This study is aimed at assessing the perception of patients of their tinnitus.

Material and Methods:

Design

This is a multi-center prospective study carried out in two tertiary health institutions in Nigeria. The study was carried out over a period of 12 months at the Ear, Nose and Throat Clinics of the University of Benin Teaching Hospital Benin City and the Kogi State Specialist Hospital, Lokoja, Kogi state both in the South-South and North-Central Geo-political zones of Nigeria.

Settings, Study participants and Procedure

Using a semi-structured Modified Tinnitus Impairment Questionnaire (THI-12) from Greimel et al. (Greimel et al 1999) a German version of the Tinnitus Handicap Inventory (THI) adapted from Newman et al (Newman et al, 1996). All consecutive adult patients who presented for the first time at these clinics with complaints of tinnitus were included in the study. Informed consent was obtained from the patients and the structured questionnaire was then administered. Those excluded from the study were all patients less than 18 years of age, those who did not give express consent and those patients who were on a follow up appointment. The information collected from patients/respondents included their biodata, presence of tinnitus, associated otological problems, associated problems or likely aetiology of tinnitus, their perception of the severity and hearing assessment using pure tone audiometer. The data obtained was analyzed using SPSS version 16.0 statistical software. The results are presented in tables and figures.

Results:

A total of 168 patients with tinnitus consented to partake in the study comprising 64(38.2%) males and 104(61.8%) females with M: F ratio of 1:1.6. The age ranged from 18-80years with the distribution as seen in figure 1, with a mean of

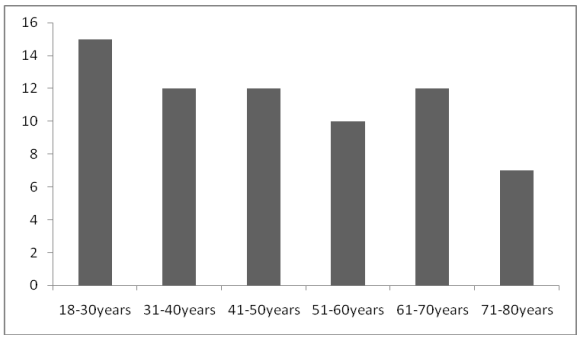

Figure 1: Age-frequency distribution chart

46.8 years (SD=17.2 ± 2.1). A majority of the respondents were self-employed in 38.1% mostly trading and artisans and the least being the unemployed in 13 (7.7%) table 1.0. About half (48.5%) of the respondents had tertiary education in both institutions, 25% had primary education and 16.2% had secondary education while 10.3% had no formal education.

The least complaint about tinnitus was among those with no formal education (10.3%),

About 32.4% complained of the tinnitus on the right side while 33.8% on the left side and another 33.8% said it was present in both ears. The duration of tinnitus varied from 3days to 25years with 16.2% of the patients presenting within one month of the onset of the symptom, while 27.9% noticed it for about 1-6months before presentation. Another 14.7% had the symptom for about 6-12 months before presentation, 10.3% presented between 12 to 24 months after the onset of the symptom and 30.9% had the symptom for over 24months.

Table 1: Occupation of the Respondents

Occupation	**Frequency**
Civil servants	41 (24.4%)
Self employed	64 (38.1%)
Students	32 (19.1%)
Unemployed	13(7.7%)
Pensioner/Retiree	18 (10.7%)
Total	168 (100%)

Table 2 shows correlation of respondents' sex with site of tinnitus indicated that there is no relationship between the sex of patient and the site of tinnitus

	Males	Females	Total
Right	27 (16.1%)	27 (16.1%)	54 (32.2%)
Left	20 (11.9%)	37 (22%)	57 (33.9%)
Bilateral	17 (10.1%)	40 (23.8%)	57(33.9%)
Total	64 (38.1%)	104 (61.9%)	168 (100%)

Chi square= 5.12, 2 degrees of freedom and P value = 0.0774

Table 3 shows symptoms and severity index with the risk factors.

Associated symptoms	
Hearing loss	106 (63.1%)
Otalgia	27 (16.1%)
Otorrhea	22 (13.1%)
Vertigo	42 (25%)
Risk factors	
Hypertension	59 (35.1%)
Diabetes	17 (10.1%)
Fever	45 (26.8%)
Loud noise	32 (19.1%)
Severity index	
Irritability	67 (39.9%)
Tired	42 (25%)
Difficult to relax	70 (41.7%)
Loss of concentration	69 (41.1%)
Uncomfortability in quiet room	64 (38.1%)
Interfere with pleasantries	32 (19.1%)
Feeling of suicide	05 (3%)
Interfere with social activities	08 (4.8%)
Interfere with other activities	30 (17.9%)
Interfere with overall enjoyment of life	47 (30%)
Interference with sleep	64 (38.1%)

Hearing loss constitute the major associated symptoms with tinnitus in 63.1% while hypertension was the commonest reported risk factor in 35.1% table 3.

On the treatment risk, 59 patients are on treatment for high blood pressure 52 were on various medications which included moduretic/methyldopa in 15, moduretic alone in 15 Nifedipine/moduretic in 10 then nifedipine alone in 7 and vasartan in 5 patients. The degree of tinnitus disturbance was found to be low in 41.2%, moderate in 44.1% high in 13.2% and no response in 1.5%.

Little effort was required to ignore the tinnitus in 66.2% of the patients, much more effort was required to ignore the tinnitus in 30.9% and no response in 2.9%.

The degree of discomfort experienced in the presence of tinnitus was said to be much in 16.2% of the respondents, little discomfort was experienced by 63.2% of the patients while 17.7% of the patients said they had no discomfort. No response was obtained in 2.9% patients.

The respondents have various degree of hearing loss however none had profound hearing loss. The degree of hearing loss found in the patients is presented in Figure 2.0 below

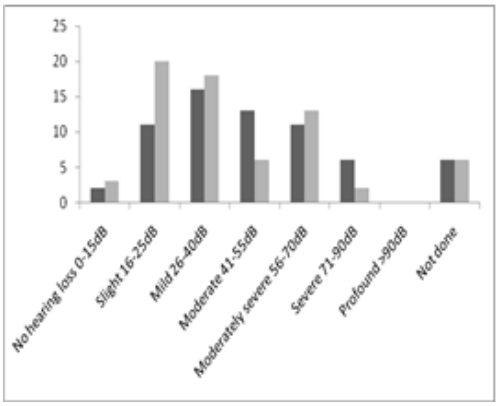

Figure 2: Degree of hearing loss

Discussion

Tinnitus is not just unwanted noise; it is extremely unpleasant and often interferes with one's concentration and attention. Previous studies have associated depression and insomnia to tinnitus severity and loudness (Former & Grist, 2000). Our study involved only adults however some reports have found tinnitus in children which is rarely expressed in our settings (Baguley & McFerran, 1999).

Prior studies about gender differences in the severity of tinnitus are inconsistent (Coles 1984, Axelsson & Ringdahl 1989). However, our study revealed that females are more affected than the males which is similar to some reports (Baguley & McFerran, 1999, Borka & Linda, 2008), but at variance with others (Alan, 2002, Folmer, 2002). Previous report of socio-demographic variables revealed that female and male individuals are often affected identically by tinnitus and that tinnitus can begin at any age, although more typically the onset occurs between the ages of 40 and 60 years (Erlandsson, 1992**)**. The average age of presentation in our study is 46.8yrs (modal age = 18-30yrs) and about 60.2% of the patients are above 40yrs which is close to the 62% quoted in another study (Coles, 1984) while 39.8% were less than 40years and this compares favourably to the 38% quoted in a similar study above (Coles, 1984). To our knowledge occupation has not been listed as a risk factor for the development of tinnitus and hearing loss except occupations which are associated with exposure to loud noise. However, our study revealed majority of our patient who presented with tinnitus are self-employed most of whom are either traders or artisans. The market place where these groups carry out their business is usually characterized by the presence of chronic noise which the patients have grown accustomed to hence, they no longer consider the environment as noisy Ologe et al 2006). The least was surprisingly found among the older people who were no longer gainfully employed with a supposedly degenerating auditory system. This may not be representative being a hospital based study, thus the need for a community based study to ascertain if there is any occupational risk for the development of tinnitus. The educational status of the respondents appears to play a role in their perception of their symptom. The evaluation of level of education showed significant differences between highly educated and less-educated people, such that highly-educated

patients exhibited higher values on the emotional-cognitive tinnitus impairment assessment. These results are in agreement with studies by Hallberg & Carlsson 1991 and Hallberg et al 1993, who observed that years of education correlated with perceived handicap in subjects with acquired hearing loss and in patients with tinnitus (Carlson et al 1993). However, it underscores the study by Entertainer et al, 2001)[25]

On the laterality of the tinnitus about 33.8% reported that the tinnitus was perceived equally on both ears while unilaterality is reported among 67.2% with 33.8% on the left side and 32.4% on the right side. This is at variance with findings in other studies that found the disturbance to be more on the left than the right side (Meilke & Walsh 1984). The correlation study showed that there is correlation between the laterality and the sex of the patient thus not significant (P value >0.05). This is at variance with another study that found a relationship (Fowler 1965, Borka & Linda, 2008).

In about 50% of the population with self-reported tinnitus, hearing from the patients' perspective have been adjudged to be normal (MRC 1981), however this is at variance with our study that found 63.2% of the patients reporting hearing loss of unspecified type as associated symptoms. Other symptoms reported are otorrhea as seen in chronic otitis media & otitis external then otalgia as seen in some patients with temporomandibular joint problems and vertigo.

Tinnitus is frequently a symptom of an associated disease process. Occasionally treatment of the disease may not relieve tinnitus, an accurate diagnosis and treatment are important to prevent additional disability. The medical conditions observed among our patients are hypertension, diabetes mellitus some of whom are on medications such as loop diuretics which may also induce or exacerbate tinnitus (Folmer, 2002).

The severity index found includes a high index of loss of concentration, irritability, loss of sleep, uncomfortability in a quiet room, inability to relax, interference with overall enjoyment, tiredness, interference with pleasantries and low index of suicidal attempt among the patients. The contribution of personality traits, such as social adjustment problems, excessive personality sensitivity or coping problems to the severity of the tinnitus perception has been suggested by some researchers in the past (Attias et al 1995).

It is important to evaluate the effect of tinnitus on daily life. Surprisingly most patient evaluated the degree of disturbance to be moderate and the perceived severity of tinnitus is related to measurements of its loudness which is at variance with other studies that found it to be unrelated (Shergill et al, 2001). The effect of tinnitus is greatest in patients who report loss of concentration, irritability, sleeplessness, and difficulty to relax and among those who are depressed, who are socially isolated, or who have psychiatric symptoms (Alan et al 2002). Treating these problems may reduce the effect of tinnitus, even though the percept is unchanged (Hunter et al 2003, Brandy & Lynn 1995). The exact prevalence of hearing loss among patients with tinnitus is high but difficult to determine. Among Patients with normal hearing, typically defined as audiometric (or hearing-level) thresholds that are 20 to 25 dB or less at frequencies ranging from 250 to 8000 Hz, it is likely that many once had better hearing or have impairments at frequencies higher than 8000 Hz (Alan et al 2002). A comprehensive audiologic evaluation is essential. To quantify any hearing loss and identify any treatable conductive component of hearing loss in the patients. However, our study revealed that most patient had hearing level on the right hear is between 26-40dB while on the left is between 16-25dB, the least Audiological findings was normal hearing and profound hearing however it is surprising to note that no patient had profound hearing loss.

In conclusion tinnitus is still a problem among the patients with both otological and non otological problems as about 16.2% described it as bothersome, the need for proper evaluation of all medical problems and the need for a community based programme to assess the degree of disability of clinical tinnitus in the population will go a long way in defining its gravity.

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CASE REPORT: A GIANT URINARY BLADDER STONE IN A FEMALE PATIENT.

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Abstract

A vesical calculus weighing more than 100g is categorized as a giant urinary bladder stone. Male preponderance for urinary bladder calculi is well known. A rare case of a giant urinary bladder calculus weighing 1200g and occurring in a female patient is reported. The stone was removed by open vesicolithotomy.

KEYWORDS: Urinary bladder; Urinary Tract Infection; Calculi; vesicolithotomy

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INTRODUCTION

Primary urinary bladder stones are not common. They are associated with malnutrition.
A giant vesical calculus is defined as a stone in the urinary bladder weighing more than 100g. Giant vesical stones are universally not common and even less common in females. Fewer than 30 reports are available in the English literature.
In Nigerians, 44% bladder calculi have been reported, while, in North America, 5% urinary calculi have been reported. There have been no such case reports for Sierra Leone. Infections seem to be a predisposing factor.
We report a case of a Giant vesical calculus seen in a Sierra Leonean female with an unusual presentation.

CASE REPORT

A 36-year-old female soldier presented at the gynaecological department of Joint Medical Unit, 34 military hospitals, Freetown, with a history of intermittent haematuria, the frequency of micturition, dyspareunia, bilateral loin pains, urgency, pain and heaviness in the vagina and dysmenorrhoea. Symptoms started nine years ago and got worse in the past six months. Past medical history reveals that patient was treated for similar symptoms but never consulted a medical doctor.

On examination, she was not pale. On vaginal examination, there was a heavy, tender mass bulging into the anterior vaginal wall. All other systemic examinations were normal. A provisional diagnosis of uterine myoma, uterine prolapse or ovarian mass and urinary bladder stone was made. Laboratory investigations including Hb, PCV, WBC & differential, serum calcium, urea, and creatinine were within normal range. Uric acid was 434.4 mmol/L. Significant urine analysis results were as follows: appearance=turbid, blood (+), protein (+) and leukocytes (++). There were no worm infestations in the stool and no signs of schistosomiasis. This was followed by pelvic ultrasound including KUB, which revealed huge bladder calculi measuring 14.7 centimetres, but the ureters and kidneys were normal (Fig.1).

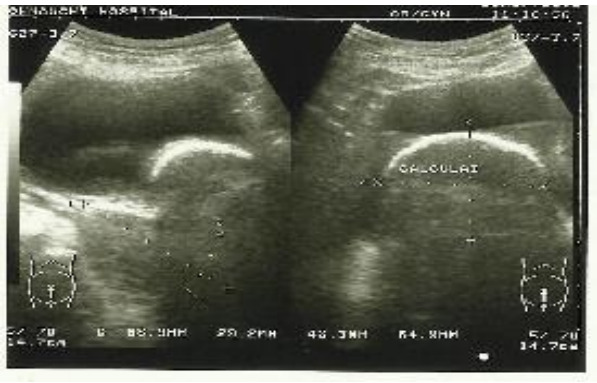

Fig. 1: UltraSound Result

Also pelvic X-ray (Fig 2) revealed a calculus. She was then referred to the surgical department for further management.

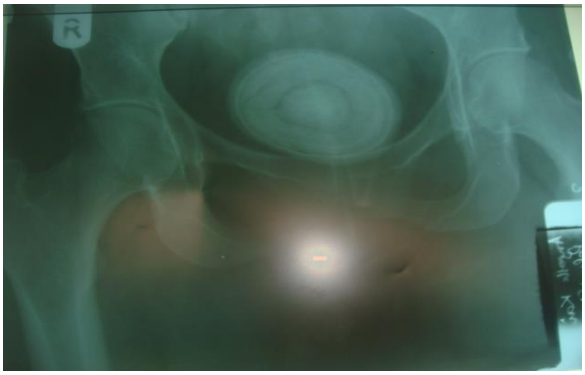

Fig. 2: Pelvic X-ray Result

In the surgical department, the patient was prepared, and surgery (Vesicolithotomy) was done two weeks later. At operation, a huge off white stone weighing 1200g and measuring 14 cm X 9 cm was removed from the bladder with minimal bleeding occupying the entire bladder (Fig.3 & 4). There was no diverticulum or ulceration seen, but the bladder wall was very thick. About 100 ml of offensive concentrated urine was drained in the urine bag during the operation. Postoperative recovery was uneventful, and she was discharged ten days later. Follow-up abdominal and pelvic ultrasound showed essentially normal upper urinary tracts.

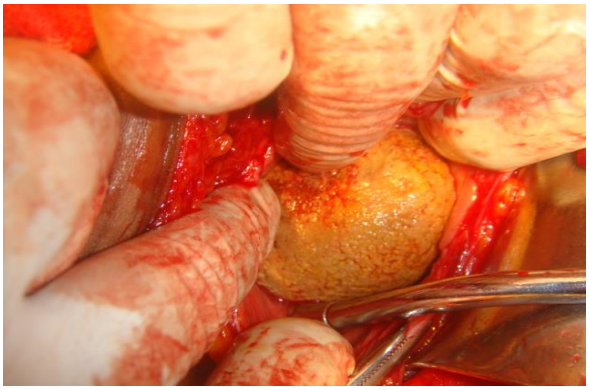

Fig 3: Huge Bladder Stone Being Removed



Fig. 4: Image of Removed Stone

The patient was followed-up for six months with no incidence.

DISCUSSION

A bladder stone, also called a vesical calculus or vesicolith, is a stone found in the urinary bladder. According to McNutt, WF 1893:185–6. , Calculus disease of the urinary system is known since historic times, Giant vesical calculi are rare in the modern era. [Becher RM *et al*, 1978a: 2272–2273]. Jan de Doot removed a bladder stone from himself according to a 1652 account in the book Observations Medicare by Nicolaes Tulp.

Primary bladder stones may occur whenever the kidneys, bladder, or ureters become inflamed. The use of urinary catheters may cause a bladder stone. Individuals who are paralyzed or are unable to pass urine adequately, may require the use of catheters. The use of these catheters may lead to an infection, which irritates the bladder, resulting in primary stone formation. A secondary kidney stone may travel down the ureter into the bladder and become a bladder stone. Frang *et al.* (1989) and Barsoum (2013) implicated digenean trematode, *Schistosoma haematobium,* in the development of vesical calculi. However, our patient was negative for worm infestations including Schistosoma. Although the stool examination was negative for schistosomiasis, the patient's original home is from Kono district which is known for high prevalence of this parasitic infection. This may be one of the possible causes or urinary tract infection as indicated from the urinalysis.

Primary urinary bladder stones account for about 5% of urinary calculi. They are usually commoner in males than in females due to obstructive uropathy [WY *et al.* 2006: 674–675:].

Urolithiasis is a disease that varies from one part of the world to the other. Some areas are grouped as high incidence areas while others are grouped as low incidence areas. Factors that determine incidence include, race, diet climatic and socio-economic status (Trinchieri ,2008). A general decrease in incidnece has been attributed to dietary and nutritional factors. Nigeria and many other sub-Saharan African countries belong to the low incidence area. (SOA *et al*. 1989: 148-61). They are more common in adults than in children. In children, primary vesical stones occur commonly in northwest India, Indonesia and the Middle East and part of China. Donald R. Smith 1981 suggest that the affected children have diets low in protein and phosphate. According to Thompson, J.C. 1921:44, secondary vesical stones develop as a complication of other urologic diseases, and 95% occur in adult men. In his report, Samison *et al.* 2002: 241-3 found that only 2 percent of primary vesical stones occurred in women

Urinary bladder stones are usually composed of calcium oxalate or magnesium ammonium phosphate (Schwart *et al.* 2000: 333-346). Majority compositions of the vesical calculi include triple phosphate, calcium carbonate, and calcium oxalate. Due to lack of analytical facilities, we were unable to do the chemical analysis of the stone of our patient

The risk factors for urinary stone formation include urinary stasis (benign prostate hyperplasia, urethral stricture, bladder neck contracture, and neurogenic bladder), urinary tract infection and foreign bodies (Mbonu O *et al.* 1984: 291-296) urine flow by lying on one side while voiding, haematuria, and even urinary outlet obstruction which lead to urinary retention, hydronephrosis, and oliguria. Our patient presented with dyspareuria, haematuria and pelvic pain. Physical examination is rarely of value in establishing a diagnosis, but instances have been cited in which a large stone was palpable on rectal, vaginal or

abdominal examination. In our patient, the stone was palpable on vaginal examination, and this was one of our provisional diagnoses which were also confirmed by IVU. Becher RM *et al 1978b*: 2272 suggests that sensing the bladder stone by feeling it "clink" on a urethral sound is an age-old technique of detecting bladder stone. Roentgenographic and sonographic studies can be helpful but the most accurate and certain means of diagnosis is cystoscopic examination,

The techniques for treatment of vesical calculi has evolved over years from 'blind' insertion of crushing forceps into the bladder to open surgical removal or extracorporeal fragmentation, cystolitholapaxy, percutaneous cystolitholapaxy and open suprapubic vesicolithotomy. However, open surgery has been the best-recommended modality for large stones (Nichols BH *et al*, 1933). Complications of this treatment include infection and damage to the bladder.

Relieving urinary stasis and eliminating infection are vital for the prevention of stone formation.

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ACUTE TOXICITY STUDIES AND ANTIDOTAL THERAPY OF ETHANOL EXTRACT OF *JATROPHA CURCAS* SEEDS IN EXPERIMENTAL ANIMALS

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Abstract

In spite of the myriad of ethno medical uses and agro-feed potential of Jatropha curcas (*JC*) seeds and the potential for production of biodiesel, toxic properties have been adduced to the plant, especially the seeds. Thus, the current study was done with the aim of investigating the toxicity of the ethanol seed extract of *JC* in rats, mice and chicks; and also to use conventional antidotes to treat intoxication in rats due to *JC* poisoning.

The LD_{50} of the ethanol extract of the *JC* seed was determined by the method initially described by Lorke. In addition, acute behavioral and CNS toxicity studies of *JC* including antidotal therapy against *JC* poisoning were done. The data was analysed using SPSS and results were expressed as mean ± SEM. $p < 0.05$ was considered significant.

The LD_{50} of IP *JC* extract ranged from 177.48 to 288.53 mg/kg (moderately toxic) for the adult female rat, adult male mouse and young male rat. For the adult male rats the LD50 values were 565.69 mg/kg (IP, slightly toxic) and >5000 mg/kg (oral, slightly toxic) and the LD_{50} of the *JC* extract for the chicks was 28.28 mg/kg (IP, highly toxic). *JC* produced a fairly dose dependent behavioral and CNS depressant effects which were reduced by atropine, EDTA and a combination of atropine, sodium nitrite & sodium thiosulphate, and EDTA. Also these antidotes either singly or in combination reduced mortality among the rats by 25-50%.

In conclusion, the ethanol extract of *JC* seeds produces behavioral changes in experimental animals possibly in part by CNS depression which were ameliorated by atropine or EDTA and a combination of antidotes. Thus, these antidotes particularly atropine, may be exploited in the management of *JC* poisoning.

Key words: Acute toxicity, LD_{50}, antidotal, behavioral indices, *Jatropha curcas.*

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INTRODUCTION

Anecdotal evidence suggests that Sierra Leone has an ancient heritage of traditional medicine and a large proportion of the population depend on it for prevention and treatment of diseases. This is however not surprising as there is currently an increase interest and demand for herbal medicine (WHO AFRO, 2008). The World Health Organization over the years had encouraged, recommended and promoted traditional herbal remedies in national health care programmes as these drugs are readily available and affordable; and the people have faith in their curative abilities (WHO AFRO, 2008).

Jatropha curcas (physic nut or purging nut) is a drought resistant shrub or tree belonging to the family Euphorbiaceae, which is cultivated in Central and South America, South-East Asia, India and Africa (Martinez-Herrera, 2006). The plant contains alkaloids, lignans, cyclic peptides and terpenes such as diterpenes. Diterpenes from different species of *Jatropha curcas (JC)* have a wide range of biological activities including tumor-promoting, irritant, cytotoxic, anti-inflammatory, antitumor, molluscicidal, insecticidal and fungicidal activities (Rakshit *et al.*, 2010). In Africa and Asia, almost all parts of *JC* including seeds, leaves, bark and roots are widely used. For instance, the seeds as well as the oil are used as purgative, and in the treatment of scabies, gout, and dropsy (Neuwinger, 1994), skin diseases such as eczema, and to soothe rheumatic pain (Heller, 1996). During the past two decades, *JC* has attracted a lot of interest particularly for its oil, which can be used for biodiesel production (Kumar and Sharma, 2008).

In spite of the myriad of ethno medical uses and agro-feed potential of *JC* seeds and the potential for production of biodiesel, toxic properties have also been adduced to parts of the plant, especially the seeds (El Badawi *et al.*, 1995). Moreover, several cases of *JC* nut poisoning in humans have been reported following accidental consumption of the seeds with symptoms of giddiness, vomiting, diarrhoea and in extreme cases death (Abdu-Aguye, 1986).

The use of *JC* for biodiesel production may lead to the widespread cultivation of *JC* and this may increase the frequency of human or animal contact with the plant, seeds, or processed products. Thus, it is imperative to investigate and document its toxicity profile. The current study was therefore undertaken with the aim of investigating the toxicity of the ethanol seed extract of *JC* in rats, mice and chicks; and also to use conventional antidotes to treat intoxication in rats due to *JC* poisoning.

MATERIALS AND METHODS

Animals

Male and female Wistar rats (150-250 g), male Swiss albino mice (15-30 g) and Shika Brown chicks - cockerels and pullets (25-50 g) were used for this study. The animals were purchased from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU), Zaria, Nigeria except the chicks which were purchased from the National Animal Production Research Institute, Shika, also of ABU. The mice and rats were housed in a room kept at 23 – 27 ^{0}C and maintained on an approximate 12 h light / dark cycle; while the chicks were kept under continuous light exposure. Clean drinking water was provided *ad libitum* to all the animals. Mice and rats were fed with standard rodent feed prepared by Vital Feeds, Jos, Nigeria; while the chicks were fed with chick mash by Pfizer, Lagos, Nigeria. The experimental design and research plan together with animal handling and disposal procedures were approved by the Departmental Animal Ethical Committee ABU, Zaria. Ethical conditions of ABU, Zaria governing the conduct of experiment with experimental animals in line with international standards, were adhered to.

Plant Identification and Extraction Process

Dried *JC* fruits were collected in May 2012 at Calaba Town in the Eastern part of Freetown, Sierra Leone and were authenticated by a Taxonomist Alhaji B.M.S. Turay, Head, Department of Pharmacognosy and Dean, Faculty of Pharmaceutical Sciences, College of Medicine and Allied Health Science.

The *JC* dried fruits were de-husked to yield the seeds which were air-dried and pulverized using pestle and mortar. 1,035 g of the *JC* seed powder was weighed and defatted by Soxhlet extraction using 1.5 L petroleum ether, for 72h at room temperature. The marcs were dried and subjected to 70% ethanol filtration for 48h. The filtrates were evaporated and concentrated to dryness on a water bath set at 50°C. The ethanol extract obtained was placed in a desiccator to dry at room temperature and later stored in an airtight container for further use. The percentage yield of the plant was 4.56%.

Acute Toxicity Studies

Determination of Median Lethal Dose (LD_{50}) of the JC extract

The Median lethal dose (LD_{50}) of the ethanol extract of the *JC* seed was determined in two phases by the method initially described by Lorke with some modifications (Lorke, 1983). The rats and chicks were divided into three groups based on gender and age (i.e. adult male rats, adult female rats and young male rats; and 5 day old cockerel, 5 day old pullets and 2 day old cockerels); and all the animals received the extract intraperitoneally except for a subset of the male adult rats which was treated orally (**Table 1**).

Table 1: Design for LD_{50} determination and behavioral studies

Expt. No.	Species	Sex	Age	Weight (g)	Route	Factor	Phase I Doses $(mg/kg)^{+}$	Phase II Doses $(mg/kg)^{++}$
1	Rat	Male	Adult	110-140	i.p.	Standard	0, 10, 100, 1000	200, 400, 800, 1600
2	Rat	Male	Adult	110-140	p.o.	Route	0, 10, 100, 1000	800, 1600, 2900, 5000
3	Rat	Female	Adult	110-140	i.p.	Sex	0, 10, 100, 1000	140, 225, 370, 600
4	Rat	Male	Young	50-70	i.p.	Age	0, 10, 100, 1000	140, 225, 370, 600
5	Mouse	Male	Adult	17-22	i.p.	Species	0, 10, 100, 1000	140, 225, 370, 600
6	Chicks	Male (cockerel)	2-day	34-39	i.p.	Species / Age	0, 1, 10, 100	5, 10, 20, 40
7	Chicks	Male (cockerel)	5-day	39-48	i.p.	Species / Age	0, 1, 10, 100	5, 10, 20, 40
8	Chicks	Female (pullet)	5-day	34-48	i.p.	Species / Age / Sex	0, 1, 10, 100	5, 10, 20, 40

N=3 per group for phase 1 study and N= 1 per group for phase 2 study except for the chicks where N=4.

A total of 48 rats, 12 mice and 48 chicks were used for the phase 1 study. Briefly, pre-determined single IP doses of *JC* extract (0, 10, 100 and 1000 mg/kg) were administered to three animals per subgroup (adult male rat, adult female rat, young male rat, and adult male mice). An additional group of 12 adult male rats (3 per subgroup) was treated orally with similar doses of the *JC* extract. Three chick per subgroup (5-day old pullet, 5-day old cockerel and 2-day old cockerel) received pre-determined single IP doses of the *JC* extract (0, 1, 10 and 100 mg/kg). The number(s) of death(s) among the animals were recorded after 24h.

The doses of the extract used in the Phase 2 study were based on the outcome of the Phase 1 study

and are shown in **table 1**. A total of 16 rats, 4 mice and 48 chicks were used in this phase and the extract was administered to 1 rat or mouse per subgroup including the orally treated rat. However, four chicks per group received single IP doses of 5, 10, 20 and 40 mg/kg respectively. The number(s) of death(s) among the animals were also recorded after 24 hours.

The LD_{50} values was calculated as follows: $LD_{50} = \sqrt{}$ the Maximum Dose of Survival x $\sqrt{}$ the Minimum Dose of Death. Using the adult male rats as standard, factors such as gender, age, specie and route of drug administration which may affect the acute toxicity of the *JC* seed extract were evaluated using the computed LD_{50} data.

Acute Behavioral Toxicity of *JC* extract

During the phase 1 LD_{50} study, an equal number of a vehicular control group for the 5 day old cockerels, adult male rats and adult male mice, was treated intraperitoneally with normal saline (NS) for comparison of the behavioural indices with their respective *JC* extract treated group. The animals were observed for behavioral changes and signs of toxicity within a 2h period. The important behavioural indices and signs of toxicity as previously described by Fugner and Hoerks (Fugner and Hoerks, 1971) were recorded. In the absence of automated activity-recording cages the behavioural indices were assessed visually by trained Research Assistants. A total of 6 Research Assistants were trained and two observed and recorded behavioral changes for 4 animals. The means of their observations were computed. Except for movement and attempted escape the other behavioral indices were qualitatively graded as follows: 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group.

Acute CNS toxicity of the *JC* extract

A total of 18 adult male Wistar rats (220-250 g) were used for this study. Under chloroform anaesthesia the head of each rat was shaved and the skin retracted, revealing the skull. The hyperstriatum (HS), optic tectum (OT) and pontine reticular formation (PRF) were implanted with stainless steel electroencephalogram (EEG) electrodes for EEG recordings. The semispinalis capitis muscle of the neck was similarly implanted with electromyogram (EMG) electrodes for EMG recordings. The electrodes were soldered to a plug and fixed over the skull with dental acrylic resin. The rats were allowed 24 hours recovery from the implantation before EEG-EMG recordings were done. Lead wires of the electrodes were connected to an EEG-EMG amplifier via a slip ring fixed above the cage allowing free movement of the rat within the cage. The amplifier was connected to the system for acquiring and processing the data. Each rat served as its own control with recording done 1h before and after drug treatment. Saline-treated controls were used to check that the instrument was functioning properly and that any detected response was not due to the procedure. The experimental rats (3 per group) were treated with a CNS stimulant amphetamine (20mg/kg), a CNS depressant chlorpromazine (50mg/kg) and ethanolic extract of *JC* seeds (0, 50, 100 and 200 mg/kg), intraperitoneally.

Antidotal therapy against acute *JC* extract toxicity in rats

Based on the anti-nutritional, heavy metal and toxic phytochemical constituents found in *JC* extract in our laboratory, and its folkloric use as a pesticide as well as the observation that respiratory failure was a constant feature of high doses during the LD_{50} study, antidotal therapy was designed against organophosphate, cyanide and heavy metal poisoning. Adult male Wistar rats used for this study were divided into six groups of 4 rats each based on weight. Rats in the first five groups were treated intraperitoneally with 10 mg/kg *JC* extract followed by IP injections of NS, atropine (20mg/kg), combination of sodium nitrite (25mg/kg) & sodium thiosulphate (1.25mg/kg), EDTA (40mg/kg), and a combination of all the antidotes respectively. Group six rats received IP NS twice, in place of the *JC* extract and the antidote.

The doses of the agents used were based on preliminary studies done with the extract, as well as previous experience with the antidotes in our laboratory. Trained Research Assistant familiar with the presentation of *JC* toxicity in rats, including respiratory distress, depression, sedation, stretching and rhythmic abdominal/thoracic muscle constriction, were used to record the signs of toxicity. The rats were observed for increased, decreased, or absence of toxicity signs 30 minutes after the administration of the antidotes, and thereafter every 30 minutes up to 2 hours. The number of deaths in each group after 24 was also recorded.

Statistical analyses and data presentation

The results were expressed as mean ± standard error of the mean (SEM) where appropriate. The data was analysed using Statistical Package for the Social Scientist (SPSS) software, version 14. Test of significance was done using one way analysis of variance (ANOVA) followed by Dunnett's Post-hoc tests for scaled variables and Kruskal-Wallis test for ranked variables. A value of $p < 0.05$ was considered significant.

RESULTS

Median Lethal Dose (LD50) of the JC extract

The LD_{50} of *JC* was determined for adult male and female rats, young male rats, adult male mice and chicks (5 day old pullet, 5 day old cockerel and 2 day old cockerel). Except for a subgroup of the adult male rats which received the *JC* extract orally, the animals in the other groups were treated intraperitoneally. Following the IP administration of the *JC* extract to the adult female rat, adult male mouse and young male rat the LD_{50} values ranged from 177.48 to 288.53 mg/kg with young male rat accounting for the least. The LD_{50} values of the *JC* extract following the IP and oral administration for adult male rats were 565.69 mg/kg and >5000 mg/kg respectively. On the other hand there was no variation in the LD_{50} value of the *JC* extract for the chicks (28.28 mg/kg) irrespective of their gender and age (**Table 2**).

Table 2: Determination of the LD50 values of *Jatropha curcas* in rats, mice and chicks

Species	Sex	Age	Weight (g)	Route	Factor	LD_{50} (mg/kg)	Classification
Rat	Male	Adult	112-142	i.p.	Standard	565.69	Slightly toxic
Rat	Male	Adult	112-142	p.o.	Route	>5000	Practically not toxic
Rat	Female	Adult	112-142	i.p.	Sex	288.53	Moderately toxic
Rat	Male	Young	50-70	i.p.	Species	177.48	Moderately toxic
Mouse	Male	Adult	17-22	i.p.	Species	288.53	Moderately toxic
Chick	Male (cockerel)	2-day	34-39	i.p.	Species/ Age	28.28	Highly toxic
Chick	Male (cockerel)	5-day	29-48	i.p.	Species/ Age	28.28	Highly toxic
Chick	Female (Pullet)	5-day	24-44	i.p.	Species/ Age/Sex	28.28	Highly toxic

The experimental animals were treated with pre-determined doses of JC extract intraperitoneally or orally in two phases for the determination of the LD_{50} of the extract. The dose of the extract used in phase was determined by the outcome of the phase 1 study. Following phases 1 & 2 treatments the number(s) of death(s) among the animals were recorded after 24 hours.

Acute Behavioral Toxicity of *JC* extract

The effect of the *JC* extract on behavioral changes and the production of toxic signs were assessed for 2 hours in the experimental animals. A vehicular control group similarly treated with NS was used for comparison of the behavioral indices. For the 5-day old cockerels no statistical significant different was noted in pecking and movement between the *JC* extract treated groups and their respective controls. The *JC* extract at the doses used produced a statistical significant increase in crouching, close eyes, sleep and respiratory distress when compared to the control groups (**$p<0.05$**) in a fairly dose dependent fashion. However, 10-100 mg/kg dose of the extract significantly reduced escape attempts when compared to their respective controls (**$p<0.05$; Table 3**).

With regards the adult male mice, the *JC* extract at the doses used produced statistical significant increase in stretching, inability to stand, sedation and respiratory distress ($p<0.05$); and statistically

significant decrease in feeding and movement (**p<0.05**) when compared to the respective controls (**Table 4**). However, with the exception of feeding which was significantly reduced in the adult male rats, at the doses used *JC* significantly increased stretching, inability to stand and respiratory distress (p<0.05), but produced no statistical significant effect on the movement of the rats when compared to their respective controls. In addition, a statistical significant increase in sedation by the adult male rats was produced by the highest dose of *JC* used (**p<0.05; Table 5**).

Table 3: The effects of ethanol extract of *JC* seeds on behavioral indices in 5-day old cockerels

Dose (mg/kg)	Pecking[1]	Movement[1]	Escape Attempts[1]	Crouching[1]	Close eyes[2]	Sleep[2]	Respiratory Distress[2]
0	2.24± 0.20	36.50±17.52	6.00±2.38	0.00±0.00	0.38±0.24	0.00 ±0.00	0.00±0.00
1	1.88± 0.20	33.17± 8.69	10.00±5.55	1.89±0.39*	1.87±0.22*	2.45±0.17*	1.67±0.22*
10	0.78±0.27	10.67± 4.76	0.00±0.00*	2.43±0.16*	2.37±0.26*	2.35±0.20*	1.44±0.35*
100	1.20± 0.48	27.17±13.47	0.00±0.00*	1.99 ±0.35*	2.34±0.22*	2.26±0.30*	1.65± 0.41*

*The cockerels were treated with JC extract (1-100 mg/kg) intraperitoneally and a vehicular control group was similarly treated with NS (0 mg/kg JC). They were observed for behavioral changes and signs of toxicity within a 2 hour period. Except for movement and attempted escape which were quantitatively assessed the other behavioral indices were qualitatively graded as follows: 0 –no effect, 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group. N=3 per group; [1]Actual numbers counted; [2]Ranking from 0 (no effect) to 3 (maximum effect); Data are means ± SEM; * P<0.05 vs. control.*

Table 4: the effects of ethanol extract of *Jatropha curcas* seeds on behavioral indices in adult male mice

Dose (mg/kg)	Feeding[1]	Movement[1]	Stretching[1]	Inability to stand[1]	Sedation[2]	Respiratory distress[2]
0 (control)	12±2.74	11.00±4.63	0.00±0.00	5.33±2.76	5.17±2.79	0.17±0.17
10	0.00±0.00*	8.33±3.51	18.33±4.36*	14.00±5.15*	17.17±3.38*	10.50±3.13*
100	0.00±0.00*	3.33±1.28*	16.33±3.44*	16.00±5.81*	21.00±2.84*	10.83±2.95*
1,000	0.00±0.00*	6.50±1.26	16.50±4.20*	19.00±3.75*	21.50±2.59*	16.17±2.57*

*The adult male mice were treated with JC extract (10-1,000 mg/kg) intraperitoneally and a vehicular control group was similarly treated with NS (0 mg/kg JC). They were observed for behavioral changes and signs of toxicity within a 2 hour period. Sedation and respiratory distress were qualitatively graded as follows: 0 –no effect, 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group. N=3 per group; 1Actual numbers counted; 2Ranking from 0 (no effect) to 3 (maximum effect); Data are means ± SEM; * P<0.05 vs. control*

Dose (mg/kg)	Feeding[1]	Movement[1]	Stretching[1]	Inability to stand[1]	Sedation	Respiratory distress

0	7.33±2.93	9.0±3.84	0.33±0.33	7.5±4.97	9.17±3.89	1.50±1.50
10	0.83±0.40*	18.67±8.74	19.67±3.05*	11.0±2.85*	12.83±3.46	9.00±3.03*
100	0.33±0.33*	24.67±5.46	25.75±2.65*	12.33±2.03*	9.50±3.03	14.17±3.39*
1,000	0.33±0.33*	26.17±9.42	19.00±2.98*	17.33±2.82*	17.17±3.91*	22.33±2.67*

Table 5: The effects of ethanol extract of *Jatropha curcas* seeds on behavioral indices in adult male rats

*The adult male rats were treated with JC extract (10-1,000 mg/kg) intraperitoneally and a vehicular control group was similarly treated with NS (0 mg/kg JC). They were observed for behavioral changes and signs of toxicity within a 2 hour period. Sedation and respiratory distress were qualitatively graded as follows: 0 –no effect, 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group. N=3 per group; [1]Actual numbers counted; [2]Ranking from 0 (no effect) to 3 (maximum effect); Data are means ± SEM; * P<0.05 vs. control.*

Acute CNS toxicity of the *JC* extract

In order to investigate the CNS effects of the *JC* extract EEG tracings of rats were separately compared with that produced by a conventional CNS stimulant and CNS depressant. Thus, adult male rats were treated intraperitoneally with amphetamine (20 mg/kg), or chlorpromazine (50 mg/kg) or varying does of *JC* (0, 50, 100 and 200 mg/kg). As expected amphetamine (20 mg/kg) and chlorpromazine (50 mg/kg) produced EEG tracings consistent of CNS stimulation and depression respectively. The CNS effect produced by *JC* was dose-dependent and similar to that of chlorpromazine (**Figure 1&2**).

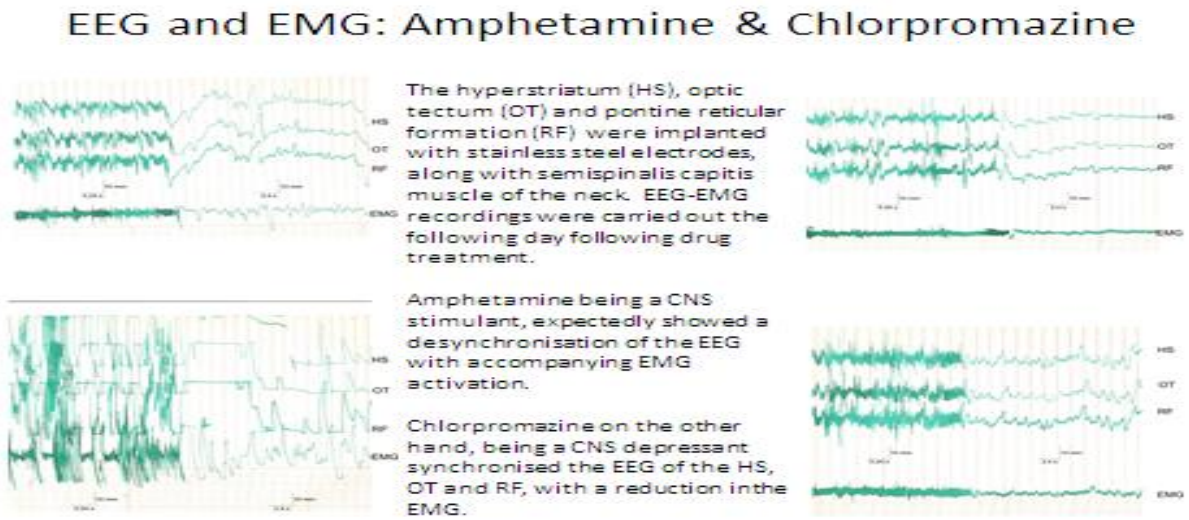

***Figure 1**: the effect of amphetamine (20 mg/kg) and chlorpromazine (50 mg/kg) on EEG and EMG recordings in rats. The rats were treated with single doses IP amphetamine (left) or chlorpromazine (right) and EEG-EMG recordings were done 24 hour later.*

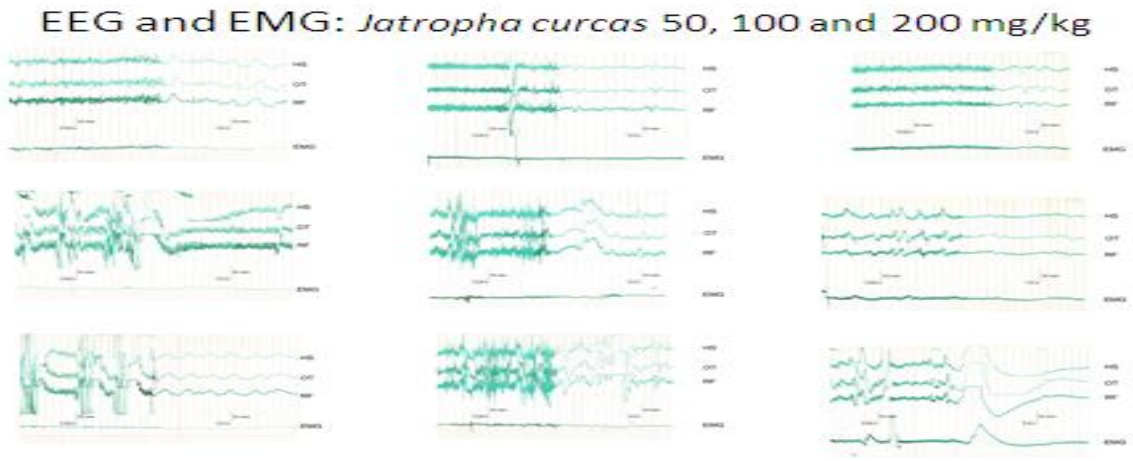

Figure 2: the effect of varying doses JC extract (50-200 mg/kg) on EEG and EMG recordings in rats. The rats were treated with single doses of 50 mg/kg JC (left), 100 mg/kg JC (center) and 200 mg/kg JC (right) and 24 hours later the EEG-EMG recordings were done.

Reduction of the toxic effect of the *JC* extract by conventional antidotes

The conventional antidotes at the doses used had no statistically significant effect on the *JC* extract toxicity up to 60 minutes (**results not shown**). At 90 and 120 minutes atropine and the combination antidotes (i.e. atropine, sodium nitrite & sodium thiosulphate, and EDTA) at the doses used significantly reduced the *JC* toxicity (**p<0.05; Figures 3A & 3B**). In addition, 40 mg/kg EDTA significantly reduced *JC* intoxication at 120 min (**p<0.05; Figure 3B**). Mortality among the rats was reduced by 50% with atropine (20 mg/kg) whilst sodium nitrite 25 mg/kg & sodium thiosulphate1.25 mg/kg, EDTA 40 mg/kg, and combination antidotes each reduced mortality by 25%.

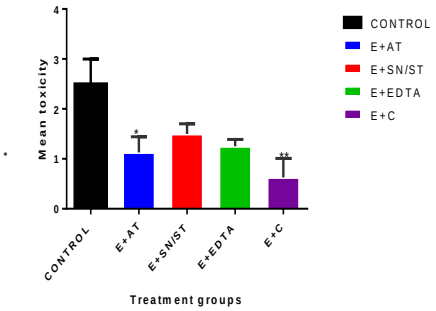

Figure 3A

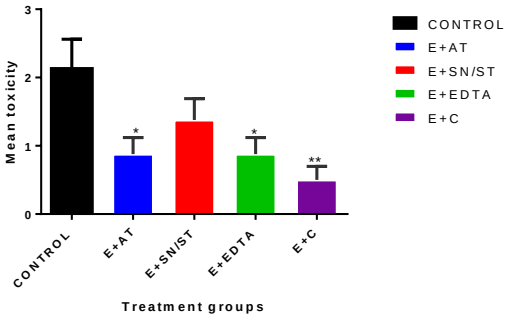

Figure 3B

Figure 3(A-B) *the effect of conventional antidotes {atropine (AT), Sodium nitrite and sodium thiosulphate (SN/ST), ethylene diamine tetra acetate (EDTA), and combination of three groups (C)} in reducing the toxic effect of JC extract (10 mg/kg). Rats were divided into five groups and were treated intrperitoneally with JC (E) only, E and Atropine (E+AT), E and Sodium Nitrite & Sodium thiosulphate (E + SN/ST), E and EDTA (E + EDTA) and E and combination of the three antidotes (E + C) respectively. The mean toxicity produced by JC (E) was recorded represented in the figure as control, and the ability of the antidotes to reduce the toxic effect of JC within (A) 30 minutes, (B) 60 minutes, (C) 90 minutes and (D) 120 minutes was noted. Data are means of triplicate observations ± SEM with n=4 per group.*P<0.05 vs. control group; **P<0.01 vs control.*

DISCUSSION

The result of the current study shows that the LD_{50} of the IP ethanol extract of *JC* for the adult female rat, adult male mouse and young male rat range from 177.48 to 288.53 mg/kg with young male rat accounting for the least; where as the LD50 values of the *JC* extract following the IP and oral administration for adult male rats were 565.69 mg/kg and >5000 mg/kg respectively. In addition, the LD50 value of the *JC* extract for the chicks was 28.28 mg/kg irrespective of their gender and age. Thus the LD_{50} values of the ethanol extract of *JC* seed showed specie, gender and age variation. According to the classification by Matsumura (1975) and Corbett and Colleagues (1984) the ethanol seed extract of the JC is moderately toxic to the adult female and young male rats and adult male mice, slightly toxic to the adult male rats, highly toxic to the chicks (Matsumura, 1975; Corbett *et al*.; 1984). These latter authors independently classified agents with LD_{50} values of less than 1, 1-50, 50-500, 500-5000, 5000-15000 mg/kg and greater than 15 g/kg as being extremely toxic, highly toxic, moderately toxic, slightly toxic, practically non-toxic and harmless respectively.

Unlike IP administration of the *JC* extract, oral administration produced practically no toxic effect to the adult male rats further demonstrating route variation. The gender and age variation in the *JC* toxicity seen in rats may account in part for the exclusion of females and young children from most therapeutic clinical trials. That no gender or age differential was noted in the acute toxicity of the extract in chicks as a specie was not surprising given that the 2-days and 5-days are so close, and below what one may term 'pubertal' when sex differences normally become evident. With respect to specie difference, chicks were more susceptible to the effects of the extract as evidenced by the least LD_{50} followed by mice and rats. The specie variation in toxicity may be relevant given that *JC* is sometimes used as a household fence, and unintentional poisoning could occur in domestic animals as well as humans. The highest susceptibility of chicks to the *JC* seed extract toxicity demonstrated in the current study is in consonance with an earlier study by El-Badawi and colleagues in 1995 in which they reported high incidence of mortality among Brown Hisex chicks fed with diets containing 0.5% *JC* seed (El-Badawi *et al.;* 1995).

Unlike the current study which reports slight toxicity of the ethanol extract of IP *JC* seeds in rats, the methanol extract of IP *JC* seeds from Nigeria was reported by Oluwole and Bolarinwa in 1997 to be highly toxic in rats with an LD_{50} value of 25.19 mg/kg (Oluwole and Bolarinwa, 1997). Furthermore, the methanol extract of *JC* stem bark and leaves from India was reported to be slightly toxic in rats following IP and oral administration with an LD_{50} of 2,000 mg/kg (Sacdeva *et al.;* 2012) and 2,500 mg/kg (Mishra *et al.;* 2012) respectively. These variations may not be unrelated to the differences in the toxic materials of *JC* from different parts of the world.

In the current study, the ethanol extract of *JC* seeds in the 5-day old cockerels produced behavioral changes such as significant increase in crouching, closing of the eyes, sleep and respiratory distress and a significant decrease in escape attempts; with decrease in food intake and uncoordinated movement. With regards the adult male mice and rats, the *JC* extract at the doses used produced significant increase in stretching, inability to stand, sedation and respiratory distress, and significant decrease in feeding. Although movement in the adult male mice was significantly reduced by the *JC* extract, it was not altered in the adult male rats. The EEG recordings in the adult male rats following the administration of amphetamine produced desynchronisation of the EEG with accompanying EMG activation consistent of CNS stimulation. Chlorpromazine on the other hand, being a CNS depressant produced a synchronized EEG pattern of the hyper striatum, optic tectum and reticular formation, with a reduction in the EMG; which is also consistent with CNS depression. The EEG recordings of the ethanol extract of the *JC* seeds showed pattern that was very similar to chlorpromazine; a CNS depressant effect which was dose dependent.

Thus the behavioral effects were fairly dose dependent and could be due to loss in skeletal muscle tone or CNS depression. The CNS depressant effects of several species of the *Jatropha* plant have been alluded to by several researchers

including Apurba and colleagues (2013), Akanmu and colleagues (2005), Wannang and colleagues (2004), and Fojas and colleagues (1986). These researchers have earlier reported EEG recordings consistent of CNS depression by extracts of *JC* leaves (Fojas *et al.;* 1986), *Jatropha gossypifolia* fruits (Apurba *et al.;* 2013), *JC* seeds (Wannang *et al.;* 2004) and *Stachtarpheta cavennesis* (Akanmu *et al.;* 2005) in rodents. Generally it is presumed that behavioral changes result from changes in the number of activated dopamine receptors. Although dopamine had been showed to be an inhibitory neurotransmitter in the brain which could produce behavioral changes (Bloom *et al*.; 1975), it had been documented to have both inhibitory and excitatory actions in different parts of the brain (Meller *et al*.; 1989, Wambebe and Osuide, 1980). Thus, the behavioral changes and CNS depressant effects produced by *JC* in this study may not be unrelated to its effects on dopamine either directly or indirectly by altering the balance between dopaminergic and cholinergic activities. The latter effect is further supported by the fact that the toxic effect of the *JC* extract in rats was ameliorated by atropine.

For the antidotal therapy studies, the signs of toxicity (decreases in food intake, movement and escape attempts; plus increases in stretching, sedation and respiratory distress) of the ethanol extract of *JC* seed at 30 and 60 min were not reduced by none of the antidotes at the doses used. However, at 90 and 120 minutes atropine and the combination antidotes (i.e. atropine, sodium nitrite & sodium thiosulphate, and EDTA) at the doses used significantly reduced the *JC* extract toxicity; whilst 40 mg/kg EDTA significantly reduced *JC* intoxication at 120 min. Mortality among the rats was reduced by 50% with atropine whilst EDTA, the combination of sodium nitrite and sodium thiosulphate, and the combination of all the antidotes, each reduced mortality by 25%.

The reduction in the toxicity produced by the ethanol extract of the *JC* seeds by atropine indicates that *JC* may possess either a direct or indirect cholinergic activity. As the ethanol extract of *JC* seeds have been shown in our laboratory to contain heavy metal it is highly probable that EDTA may have reduced the toxicity by *JC* by chelation of heavy metals. Additionally, *JC* was shown in our laboratory to contain cyanide and most of the rats which presented with signs consistent of cyanide toxicity died. Although the combination sodium nitrite-sodium thiosulphate did not significantly diminish signs of *JC* toxicity it reduced mortality among the rats by 25% suggestive of cyanide involvement.

In conclusion, the ethanol extract of the *JC* seeds produces behavioral changes in experimental animals possibly in part by CNS depression and these effects are ameliorated by atropine or EDTA and a combination of antidotes. Thus, these antidotes particularly atropine, may be exploited in the management of Jatropha poisoning in animals and humans.

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HAEMATINICS AND WEIGHT REDUCTION PROPERTIES OF ETHANOL EXTRACT OF JATROPHA CURCAS SEEDS IN RATS

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ABSTRACT

Despite the potential uses of Jatropha curcas (JC) very little is known about its effects on the blood cells. The current study was therefore undertaken to investigate the effect of JC seed from Sierra Leone on different haematological parameters.

Ethanol extract of JC was administered daily to adult male and female Wistar rats (120-200g) either intraperitoneally or orally for 3 days or 7 days respectively. For each treatment route the rats were divided into six sub-groups as follows: group 1 rats (control group) received 0.9 % physiological saline (i.p.) or de-ionised water (p.o.) at 10 ml/kg, while rats in groups 2, 3 and 4 received 50, 100 and 200 mg/kg dose of the extract respectively; and groups 5 and 6 rats received iron (5 mg/kg) and EDTA (40 mg/kg) respectively. The rats were weighed before and after the treatment period, and at the end of the treatment period blood samples were collected by cardiac puncture. The following haematological parameters were evaluated: RBC, WBC, lymphocytes, neutrophil counts, Hb, HCT concentrations (with MCV, MCH, MCHC values calculated). Data obtained were analysed by one way ANOVA using the statistical software Graph Pad Prism version 6; with $p<0.05$ taken to be statistically significant.

The extract decreased the body weights of the rats in a dose, route and sex dependent manner; with i.p. > p.o., male > female. The extract also significantly ($p<0.05$) increased the mean Hb, HCT, MCV, MCH, MCHC, RDW values and neutrophil counts; while WBC and lymphocyte counts were decreased by the extract. The aforementioned increases in the mean Hb and RBC-related indices were to a large extent dose-dependent, with peak effects recorded at 100 mg/kg. It was concluded that J. curcas seeds could be exploited for haematinics benefits, provided its detailed pharmacokinetic and safety profiles have been documented.

Key words: Haematinics, Plant extract, Jatropha curcas, Wistar rats, Weight, Obesity.

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INTRODUCTION

Sierra Leone, like many other African countries, has a diverse healthcare system with traditional medicines forming part of the primary healthcare, though informally. It is known that there is a strong coexistence between western medical system which is based on modern scientific medicine and a variety of non-conventional therapies, including a multiplicity of local indigenous systems founded on traditional beliefs and practices. African traditional healing forms part of African culture and today traditional healers remain essential for the health and well-being of a large proportion of the population particularly the black population (Van der Linde, 1997). A survey conducted by WHO in 2008, indicated that about 80% of the Sierra Leonean population rely on, and resort to traditional medicine practitioners (WHO AFRO, 2008).

Jatropha curcas is one of several species of Jatropha plants cultivated globally including across West Africa. The plant Jatropha curcas (JC), produces seeds that are a source of biodiesel and also contain several metabolites of pharmaceutical importance (Okoli et al., 1994). For instance the seeds have been shown to be useful in the management of dropsy, gout and paralysis, and the seed oil have been applied to treat skin diseases such as eczema and to soothe rheumatic pain (Okoli et al., 1994). Okoli and colleagues in their publication concluded that the linoleic acid content in JC seed oil could be of interest for the skincare industry (Okoli et al., 1994). Despite these and other potential uses of JC seeds and other parts of the plant, the effects of JC on the blood cells have not been investigated and documented.

Blood being a specialized body fluid that supplies the tissues with nutrients, oxygen and removes harmful waste is composed of red blood cells (RBCs), white blood cells (WBCs) and platelets. Anaemia which is characterised by low levels of RBCs, and therefore lack of oxygen-carrying capability of the blood, is the most common haematological disorder globally. It is particularly a very big problem in Sierra Leone affecting at least 45% women and young children (DHS, 2008). Deficiency of WBCs (defence system against bacterial and viral infections) can lead to many immune diseases such as rheumatoid arthritis, German measles or rubella (Liang, 1999). Derangement of platelet levels affects blood clotting while those of serum electrolyte destabilizes homeostasis.

Since JC is known for its extensive traditional uses, it may be a good precursor for a potential drug source with known safety profiles (Gadir et al., 2003). As there are known variations in the composition of JC from across the world the effect of JC therefore varies in different parts of the world (King et al., 2009). Moreover, based on the relevance of the blood and the potential uses of JC seed, it is clear that investigations into the effect of JC seed on haematological indicators, will address a clear research need. Thus as very little has been published or reported on the haematological effect and toxicity of the seeds, the current study aims to investigate the effect of JC seed from Sierra Leone on the different haematological parameters and to establish whether JC has any haematinics property.

MATERIALS AND METHODS

Experimental Animals

A total of one hundred and twenty Wistar rats (120-200g) of both sexes purchased from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the study. The animals were allowed free access to standard rodent diet (brand) from vital feeds and clean drinking water throughout the experimental period. They were housed in cages (about 12 to a cage) in a room kept at room temperature which was relatively humid. Male rats were kept in cages separate from the female rats to avoid the females getting pregnant during the experimental period. The animals received humane care and the study protocol was in compliance with the university's guidelines for the use of laboratory animals. The experimental protocol was approved by the Ahmadu Bello University's Ethics Committee.

Plant extraction process

Dried JC fruits were collected on the 21st. May 2012 at Calaba Town in the Eastern part of Freetown, Sierra Leone and were authenticated by a Taxonomist Alhaji B.M.S. Turay, Head, Department of Pharmacognosy and Dean, Faculty of

Pharmaceutical Sciences, College of Medicine and Allied Health Science.

Jatropha curcas fruits were de-shelled to obtain the seeds and were pulverized using a mortar and pestle. 350.04g of the plant material was defatted for 72 hours at room temperature via Soxhlet apparatus using petroleum ether (60-80°C) as the extraction solvent. The petroleum ether extract was evaporated using a heating mantle and the oil was collected and weighed.

The defatted marc was dried at room temperature for 4 hours, placed in a separating funnel for cold extraction using 1L of 70% ethanol for 48 hours. The ethanol extract was collected in a beaker, the marc rinsed with 500 mL of 70% ethanol and collected as well. Two crucibles were weighed and the filtrate was poured into them. These crucibles containing the filtrate were heated in a water bath at 55°C to evaporate the marc filtrate. Thereafter, the crucibles were weighed and the ethanol extract stored in an airtight container for future use. The extract came out as semi-solid dark brownish paste-like material with a total yield of 14g of the dried extract (4% of the plant material).

Dosing of the experimental animals

The ethanol extract of JC was administered to adult male and female (120-200g) wistar rats either intraperitoneally or orally. For each treatment route of drug administration, the rats were subdivided into six groups.

Intraperitoneal treated Rats

A total of 72 adult Wistar rats (120 -200g) of both sexes were treated via intraperitoneal (IP) route and were divided into 6 sub-groups. The JC extract dissolved in normal saline (NS) was administered daily as a single dose to the first, second and third groups of rats at 50, 100, and 200 mg/kg respectively for three days. The fourth group (control group) received equal volume of the NS daily for three days; whilst the fifth and sixth groups received single daily doses of iron (5 mg/kg) and EDTA (40 mg/kg) respectively for three days.

Orally treated Rats

A total of 48 adult Wistar rats (120 to 200 g) of both sexes received single daily doses of the extract and the other drugs via oral route daily for 7 days. They were also divided into six groups and treated orally as described above.

Haematological studies

At the end of the experimental period, the rats were anaesthetized using light chloroform and blood was collected from each rat by cardiac puncture into labelled heparinised bottle. The blood samples were taken to the haematology department of the Sick Bay at Ahmadu Bello University and the following haematological parameters were determined using an auto analyser: RBC count, MCV, RDW, MCH, MCHC, Hb, HCT or PCV; Platelet count; and WBC and differential count.

In addition to the haematological analysis, the rats were weighed prior to administration of JC extract and other experimental drugs (day 0) and at the end of the experimental periods, day 4 for IP route and day 8 for the oral route. The weights of the rats at the end of the experimental periods were compared to day 0 for the two routes.

Statistical analysis

Results are expressed as mean ± SEM. Means were obtained from multiple experiments performed in triplicates. Data were analyzed and graphed with the commercially available statistical software graph pad prism version 6 (Graphpad software, San Diego, CA, USA). Differences between mean values within the groups were determined by one way analysis of variance (ANOVA) followed by Dunnett's (post hoc) test for comparison of multiple means. Differences between mean values for different groups were tested for using the unpaired, two-tailed Student's t-test. The level of significance was set at $p<0.05$.

RESULT

Body weight of the Rats

IP administration of JC (50, 100 and 200 mg/kg) to the rats produced a dose dependent reduction in their body weights; and the reductions were statistically significant at 100 and 200 mg/kg ($p< 0.05$; Figure 1A). However, when the rats were disaggregated by sex, statistically significant reductions in weight were still noted with 100-200 mg/kg dose of the extract for the males ($p<0.05$; Figure 1B) but only 200 mg/kg dose of JC produced a significant reduction in body weight for the female rats ($p<0.05$; Figure 1C). It was also found that iron and EDTA at the doses used had no effect on body weights of the rats irrespective of the route of administration {Figures 1(A-C)}. Unlike the IP route, when similar doses of the JC extract were administered to the rats daily for 7 days orally, there were no statistical significant effects on their body weights (result not shown).

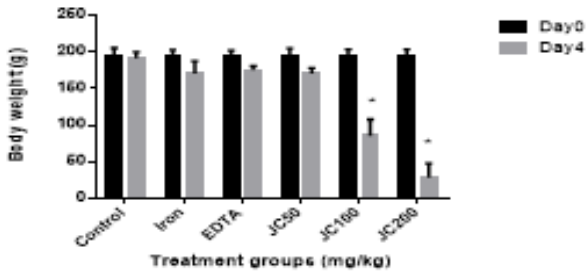

Fig. 1A

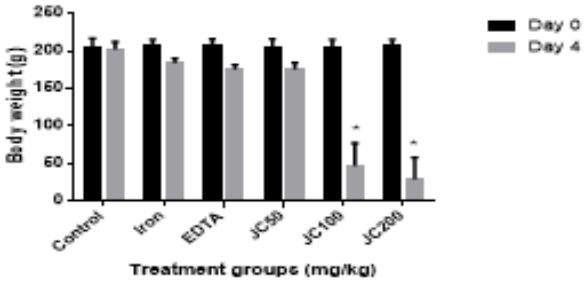

Fig. 1B

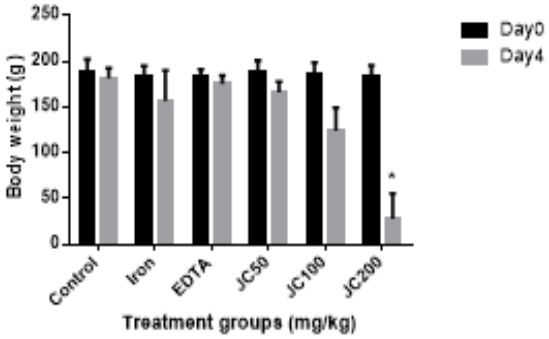

Fig. 1C

Figure 1(A-C): The effect of ethanol extract of JC on the mean body weight of rats (A) both sexes, (B) males and (C) Females. The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) intraperitoneally for 3 days and their mean weight computed on day 4. N=8 for each sub-group. * $p< 0.05$ vs. control group.

Haematological Indices

Hematological parameters such as RBC, MCV, RDW, MCH, MCHC, Hb, HCT, Platelets, WBC and differential count (neutrophils and lymphocytes) were determined in each treatment group after IP and oral administration of the experiment dugs on days 4 and 8 respectively.

Irrespective of the route used, the doses of JC extract administered had no statistical significant effect on the RBC count of the rats when compared to the control group ($p>0.05$; Tables 1-2). Nevertheless, following the IP administration of the JC extract a fairly dose dependent increase in Hb, HCT, MCV and MCH was noted up to the 100 mg/kg dose. The increase produced by the 100 mg/kg dose of the JC extract was found to be statistically significant ($p<0.05$; Table 1). Increase in the dose of the extract to 200 mg/kg did not produce further increase in these RBC parameters. Furthermore, 100 mg/kg IP dose of the JC extract significantly reduced platelet count when compared to the control group ($p<0.05$; Table 1). With regards to WBC and differential counts, the extract

administered via IP route at 50 mg/kg, significantly reduced WBC and lymphocytes but significantly increased neutrophils when compared to their respective controls ($p < 0.05$; Table 1).

A statistical significant increase in Hb, HCT, MCV and MCH were noted in rats treated via the IP route with 5 mg/kg iron and 40 mg/kg EDTA when compared their respective control groups ($p<0.05$; Table 1). The platelet count was significantly reduced by IP administration of 5mg/kg dose of iron ($p<0.05$) whereas IP administration of 40 mg/kg EDTA had no significant effect on the platelet count (Table 1).

Unlike the IP administration of the JC extract, oral administration produced no statistically significant effect on the haematological parameters of the rats when compared to the control groups (Table 2) except for MCH count, which was significantly reduced by the JC extract at 50 mg/kg (15.55 ± 2.23) and 100 mg/kg (16.00 ± 2.31) dose ($p<0.05$; Table 2). Furthermore, with the exception of MCH which was significantly reduced by the oral administration of 40 mg/kg EDTA ($p < 0.05$), oral EDTA and iron at the doses used had no significant effect on the haematological parameters of the rats (Table 2).

Table 1: The Effect of IP Ethanol Extract of JC on Haematological Indices in Rat

Parameters	Control	JC 50 mg/kg	JC 100 mg/kg	JC 200 mg/kg	Iron	EDTA
RBC ($X10^6/\mu L$)	8.00 ± 0.16	7.67 ± 0.25	8.19 ± 0.24	7.31 ± 0.00	8.38 ± 0.30	7.32 ± 0.43
HCT (%)	42.18 ± 0.23	45.45 ± 1.44	49.69 ± 1.54	40.60 ± 0.00	49.40 ± 1.86	43.24 ± 1.84
Hb (g/dl)	12.93 ± 0.09	14.41 ± 0.35	15.65 ± 0.39	13.10 ± 0.00	15.29 ± 0.47	13.65 ± 0.51
MCV (fl)	52.89 ± 1.13	59.38 ± 0.73	60.66 ± 0.88	55.50 ± 0.00	58.89 ± 0.38	59.45 ± 1.07
MCH (pg)	16.25 ± 0.44	18.89 ± 0.32	19.11 ± 0.21	17.90 ± 0.00	18.26 ± 0.11	18.84 ± 0.45
MCHC (g/dl)	30.65 ± 0.18	31.76 ± 0.25	31.55 ± 0.25	32.30 ± 0.00	30.99 ± 0.31	31.60 ± 0.25
PLT ($X10^3/\mu L$)	646.38 ± 9.48	622.50 ± 72.13	393.50 ± 29.07	474.00 ± 0.00	521.75 ± 33.66	636.88 ± 41.03
WBC ($X10^3/\mu L$)	16.73 ± 1.07	8.90 ± 0.44	9.84 ± 0.80	15.50 ± 0 .00	12.99 ± 2.00	11.44 ± 1.36
Lymphocytes ($X10^3/\mu L$)	87.10 ± 0.94	64.69 ± 3.03	52.64 ± 6.12	29.70 ± 0.00	70.73± 1.71	67.66 ± 4.83
Neutrophils ($X10^6/\mu L$)	12.90 ± 0.94	35.31 ± 3.03	47.36 ± 6.12	70.30 ± 0.00	29.28 ± 1.71	32.34 ± 4.83

*The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) intraperitoneally for 3 days and their RBC count, HCT, Hb, MCV, MCH, MCHC, WBC and differential count, and platelets were determined on day 4. N=8 for each sub-group and results were expressed as mean ± SEM. * $p < 0.05$ vs the control.*

Table 2: The Effect of Oral Ethanol Extract of *JC* on Haematological Indices in Rat

Parameters	Control	JC 50 mg/kg	JC 100 mg/kg	JC 200 mg/kg	Iron	EDTA
RBC ($X10^6/\mu L$)	7.11 ± 0.16	6.59 ± 0.95	6.32 ± 0.92	7.27 ± 0.42	7.58 ± 0.18	6.91 ± 1.04
HCT (%)	43.25 ± 0.52	37.86 ± 5.50	36.61 ± 5.31	42.34 ± 2.05	45.03 ± 0.75	40.91 ± 6.14
Hb (g/dl)	13.48 ± 0.13	11.70 ± 1.69	11.51 ± 1.66	13.34 ± 0.60	13.89 ± 0.21	12.56 ± 1.86
MCV (fl)	57.13 ± 3.86	50.26 ± 7.20	50.79 ± 7.30	58.44 ± 0.96	52.36 ± 7.50	51.83 ± 7.41
MCH (pg)	24.28 ± 5.29	15.55 ± 2.23	16.00 ± 2.31	18.45 ± 0.40	18.34 ± 0.26	15.98 ± 2.29
MCHC (g/dl)	29.69 ± 1.58	27.06 ± 3.87	27.55 ± 3.95	31.68 ± 0.16	29.60 ± 1.31	27.09 ± 3.88
PLT ($x10^3/\mu L$)	744.75 ± 38.87	594.50 ± 92.89	690.50 ± 104.90	606.50 ± 33.86	730.38 ± 53.57	595.38 ± 99.23
WBC ($x10^3/\mu L$)	17.09 ± 2.80	16.83 ± 3.30	12.54 ± 2.20	14.76 ± 2.76	21.61 ± 2.59	15.83 ± 2.88
Lymphocytes ($X10^3/\mu L$)	85.43 ± 1.50	74.26 ± 10.79	64.51 ± 9.71	64.78 ± 10.80	82.34 ± 0.75	71.19 ± 10.21

*The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) orally for 7 days and their RBC count, HCT, Hb, MCV, MCH, MCHC, WBC and differential count, and platelets were determined on day 8. N=6 for each sub-group and results were expressed as mean ± SEM. * $p < 0.05$ vs the control*

The rats were treated with NS, JC (50, 100 and 200 mg/kg), iron (5 mg/kg) and EDTA (40 mg/kg) intraperitoneally for 3 days and their RBC count, HCT, Hb, MCV, MCH, MCHC, WBC and differential count, and platelets were determined on day 4. N=8 for each sub-group and results were expressed as mean ± SEM. * $p < 0.05$ vs the control.

DISCUSSION

The JC seed extract significantly reduced the body weights of the rats in a dose and route dependent manner. This was evidenced by a progressive and significant reduction in body weight following IP treatment of the rats with varying doses of the JC extract (50, 100 and 200 mg/kg). A greater reduction in the body weights of the male rats was produced by the JC extract when compared to their female counterparts, indicating a gender dependent reduction. However, at the end of the experimental period, three of the female rats were found to be pregnant, which may have accounted for the gender variation. This may also explain in part why females are excluded from most clinical trials as pregnancy may influence the outcome of most studies or the drug may influence the pregnancy.

The route dependent difference in weight reduction may be due to the differences in the pharmacokinetic profile following IP and oral administration such as, incomplete absorption, hepatic first-pass effect and therefore bioavailability. Since the pharmacokinetic profile of the JC extract has not been fully documented, it is plausible that the oral bioavailability of the JC extract was reduced due to a combination of factors. Furthermore, one would have expected the protracted oral administration of the extract to reduce the mean body weight of the rats. However, this was not the case, possibly because the serum concentration achieved and/or the exposure period following oral administration was not sufficient enough. In a separate and independent study in our laboratory by our group, in which phytochemical analysis of the JC extract was conducted, JC was shown to contain tannins which significantly reduce food intake (Glick, 1981 and Frutos et al., 2004). Thus, the reduction in body weights may not be unrelated to the presence of tannins in the extract and therefore the reduction of food intake by the rats. Unlike the JC extract, the conventional haematinic (iron) and the known chelating agent (EDTA) at the doses used had no effect on the body weights of the rats irrespective of the route of administration.

The results also show that IP administration of the JC extract had no effect on RBC count, increased RBC indices and neutrophils, but reduced WBC and lymphocytes. The RBC count was not affected partly because the balance between the rate of production and destruction of the RBC was not altered (Adebayo et al., 2010). Although the extract had little or no effect on the RBC count, it significantly increased Hb, HCT, MCV, MCH, MCHC and RDW levels. Since MCV, MCH and MCHC are all related to individual red blood cells whilst Hb and HCT are associated with the total population of red blood cells in a sample, it is possible that the extract increases the production of the red cells which was nullify by a concomitant increase in the destruction of the RBCs. Hence there was no difference in the total RBC counts. Furthermore, these parameters were progressively increased by the extract up to a dose of 100 mg/kg. Thereafter, a further increase in dose did not increase the levels of these parameters. Thus, in this study, the optimal dose for increasing these parameters is 100 mg/kg. The effects produced by the extract on the RBC indices were similar to that produced by iron which is a conventional haematinic. Since anaemia is characterized by reduced haemoglobin, and or haematocrit levels (Provan, 1999), the extract could be a very useful haematinic agent in correcting anaemia. Unlike iron and the extract, EDTA had no effect on the RBC indices.

J. curcas extract reduced WBC and lymphocytes, but increased neutrophil counts. JC extract was shown in a separate and independent study in our laboratory by our group to contain phytochemicals such as saponins, tannins, flavonoids and alkaloids which have deleterious effects in animals (Adedaop and Abatan, 2005; Miles et al., 1993). Although the effects of these phytochemicals on WBC counts and its differentials have not been documented, the reduction of WBC and lymphocyte counts may not be unrelated to the direct or indirect actions of these phytochemicals. Since JC extract is useful in the management of several disease conditions such as arthritis, parasitic skin diseases (Heller, 1996), jaundice (Oliver-Bever, 1986), treatment with JC product may elevate neutrophil level leading to a false impression of bacterial infection. Furthermore, Swenson and Reece (1993) noted that toxic plants do not produce a direct effect on white blood cells

such as neutrophils and lymphocytes, thus the alterations in WBC and its differentials may not necessarily be due to the direct toxic or harmful effect of the extract. In addition, the effect of the known chelating agent (EDTA) and the conventional haematinic (iron) on WBC and its differentials were similar to those produced by the extract.

With regards to the platelet counts, the extract produced a dose dependent reduction in platelet counts after IP administration. Although the effects of the extract on bone marrow suppression and platelets reduction have not been documented, this reduction may not be unrelated to the phytochemicals contained in this extract.

In concluding, the study shows that ethanol seed extract of JC increases HCT, Hb and other RBC parameters except RBC count. These haematinic properties may be exploited in the management of anaemia provided its safety profile particularly on platelet count is well investigated and documented. In addition, the weight reduction property of this extract could also be explored in the treatment of obesity.

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The High Dependency Unit in the Management of Critically Ill Obstetric Patients in Low Resource Countries.

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Abstract

The care of the obstetric population requiring critical care at the intensive care unit is associated with challenges that have made the establishment of obstetric high dependency units (HDU) a priority in the developed unlike low resource countries.

The objective was to evaluate the need to establish obstetric HDUs in low resource countries.

The study was a retrospective descriptive study of obstetric patients admitted into the intensive care unit of the University of Ilorin Teaching Hospital from 1st January 2010 to30th June 2013. Those that were suitable for management at a HDU were compared with those who needed ICU care. The statistical analysis was with SPSS version 20 with p-values, x2 and odds ratio; p value <0.05 was considered significant.

All the 52 patients were postpartum; 16(30.8%) were suitable for HDU care. Of the HDU eligible patients, 11(68.8%) were of low social class, 12(75%) were booked, 11(68.8%) had no further complication at ICU and mortality was 1(6.25%). Compared to those who needed ICU care, there were no statistical significance in maternal age, parity, duration of ICU admission and total cost of ICU care. There were more cases with statistical significance of organ involvement (p<0.001), severity of Glasgow coma score at admission (p<0.001), further complications at ICU (p<0.001) and maternal mortality (p<0.001) among those needing ICU care compared to those needing HDU care.

In conclusion, establishment of the HDU will reduce cost and the burden on the few ICUs in low resource countries without increasing maternal mortality.

Keywords: High dependency unit, critically ill obstetric patients, low resource countries

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Introduction

Globally, over half a million women die annually as a result of complications of pregnancy, many more suffer varying degrees of acute maternal morbidities resulting in critical maternal illness necessitating critical care at the Intensive Care Unit (ICU) during pregnancy or puerperium. Obstetric

patients requiring intensive care range from 0.2 to 0.4% of all deliveries (Anwari et al., 2004; Lapinsky et al., 1997; Kilpatrick et al., 1992) and constitute 0.4 to16.0% of all admissions into the ICU (Anwari et al., 2004; Lapinsky et al., 1997; Kilpatrick et al., 1992; Pollock et al., 2010; Rios et al., 2012). Interventions at the ICU include therapeutic interventions like antihypertensive, inotropic agents and magnesium sulphate; arterial line insertion, central venous pressure monitoring and mechanical ventilation among others. A major limitation in the low resource countries is the limited availability of ICUs and non-availability of spaces for obstetric patients since most of the ICUs are multispecialised admitting patients from all medical specialties. This is coupled with the higher cost of ICU management in relation to the endemic poverty among the populace with resource-challenged hospitals.

This brings to the fore the role of the obstetric High Dependency Units (HDU). These are higher levels of care which lie in between a general ward and ICU care (NHS 1996); it is suitable for providing basic respiratory and single organ support. It may be as small as a three bedded unit with multidisciplinary staffing (midwifery, obstetric, anesthesia) providing invasive monitoring but usually no mechanical ventilators. It has been reported that a HDU can cater for the need of at least half of the obstetric population in need of critical care and also help in cost reduction since a major issue in ICU care is the higher cost (Zeeman 2006). Therefore, there is the need to evaluate the need for HDU services in low resource countries in order to provide easier access to obstetric patients requiring critical care.

The objective of this study was to evaluate the need for the establishment of obstetric HDUs in low resource countries like Nigeria.

Materials and Methods

The study was a total population study of all obstetric patients admitted at the Intensive care unit (ICU) of the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria from 1st January 2010 to 30th June 2013. The ICU is a multispecialised four-bedded unit with facilities for multiparameter monitoring and functioning mechanical ventilators. It receives patients from all medical and surgical units in the hospital as well as referrals from other centers.

The ICU admission register was screened and a list of all obstetric patients admitted during the study period was compiled; the case files were retrieved from the medical records department of the hospital for analysis.

The inclusion criteria were admission in the ICU during pregnancy or within 42days of its termination and the case files must be available for review. Exclusion criteria were non obstetric patients and obstetric patients whose case files were not available for review.

For this study, a HDU was defined as a critical care unit that provides all care in the ICU except mechanical ventilation. The participants suitable for HDU management were identified based on the above definition and referred to as HDU eligible and compared with those who actually required ICU care referred to as ICU eligible. The data used for this study were obtained from an audit of management of critically ill obstetric patients at the ICU of this hospital.

The data obtained included socio-demographic and obstetric parameters, indication and clinical state at ICU admission, care, complications, duration and final outcome of ICU admission. Data analysis was by using the SPSS version-20 and p-values, chi-square and odds ratio with 95% confidence interval were calculated; p value <0.05 was termed significant.

The study was approved by the UITH ethical board, sponsorship was by the researchers and there was no conflict of interest in the conduct of the study.

Results

A total of 52 case files were available for review; of these, 36(69.2%) were ICU eligible while 16(30.8%) were HDU eligible. Table 1 showed that the mean age of ICU and HDU eligible patients were 29.36±5.561 and 28.22±5.045 (p=0.766), the mean

parities were 2.00±1.585 and 1.88±1.628 (p=0.796). Also, there were no statistical significance in the booking status (p=0.131, OR 0.333, CI 0.090-1.231), level of education (p=0.232, OR 2.333, CI 0.696-7.823) and social class (p=0.506, OR 0.629, CI 0.168-2.346) of the participants.

The indications for admission into the ICU are presented in Table 2; the pattern was similar among both the ICU and HDU eligible patients with massive postpartum haemorrhage (11(30.6%) vs. 8(50%); p 0.4913) and severe preeclampsia/ Eclampsia (11(30.6%) vs. 4(25%); p 0.0707) as the two leading indications for ICU admission.

Table 3 shows the presentation, care and outcome of ICU admission. From it, 10(27.8%) ICU eligible 28(77.8%) of ICU eligible and 15(93.8%) HDU eligible were hypoxic at ICU admission (p=0.245, OR 0.23, CI 0.01-2.20). None of the HDU eligible had systolic blood pressure <90mmHg, 16(44.4%) ICU eligible and 10 (62.5%) HDU eligible had blood pressure 90-139mmHg (p=0.1472, OR 1.63, CI 1.20-2.20) while 15 (41.7%) ICU eligible and 6(37.5%) HDU eligible had systolic blood pressure ≥140mmHg (p=0.4768, OR 0.64, CI 0.16-2.58). In addition, 34(75.6%) ICU eligible and 11(68.8%) HDU eligible had organ dysfunction at ICU admission (p=0.023) while 31(86%) ICU eligible and 5(31.2%) HDU eligible had further complications at the ICU (p=<0.001). The mean durations of admission were 3.47±2.613 for ICU eligible and 3.56±3.966 for HDU eligible (p=0.923). The mean total cost of ICU admission in Naira were 48,347.22±35,370.25 for ICU eligible and 34,812.50±24,296.00 for HDU eligible (p=0.171)

Table 1: Socio-demographic characteristics of ICU and HDU eligible obstetric patients

Parameter	ICU eligible n=36 (%)	HDU eligible n=16 (%)	t-test	x^2	p-value	OR	95%CI
Mean age	29.36±5.561	28.88±5.045	0.299		0.766		
Mean parity	2.00±1.586	1.88±1.628	0.260		0.796		
Social class							
High	8(22.2)	5(31.2)					
Low	28(77.8)	11(68.8)		0.481	0.506	0.629	0.17-2.35
Booking status							
Booked	18(50.0)	4(25.0)					0.09-1.23
Unbooked	18(50.0)	12(75.0)		2.836	0.131	0.333	
Level of education							
None	13(36.1)	2(12.5)		1.54	0.3574	3.250	0.37-34.11
Primary	8(22.2)	4(25.0)		0.22	0.9999	1.000	0.11-8.93
Secondary	6(16.7)	3(18.7)		0.26	0.6913	1.560	0.21-11.93
Tertiary	9(25.0)	7(43.8)					

Table 2: Indications for admission into the intensive care unit

Indication	ICU eligible	HDU eligible	Sub total		
	n = 36 (%)	n= 16 (%)	n (%)	$_{\chi}2$	P value
Massive postpartum hemorrhage	11 (30.5)	8 (50.0)	9 (100.0)	0.474	0.4913
Severe preeclampsia/ Eclampsia	11 (30.5)	4 (25.0)	15 (100.0)	3.267	0.0707
HELLP syndrome	2 (5.6)	1 (6.2)	3 (100.0)	0.333	0.5637
Amniotic fluid embolism	2 (5.6)	0 (0.0)	2 (100.0)	-	-
Puerperal/ post abortion sepsis	2 (5.6)	1 (6.2)	3 (100.0)	0.333	0.5637
Uterine rupture	4 (11.0)	2 (12.6)	6 (100.0)	0.667	0.4142
Complication of unsafe abortion	2 (5.6)	0 (0.0)	2 (100.0)	-	-
Peripartum Cardiomyopathy	2 (5.6)	0 (0.0)	2 (100.0)	-	-

Table3: Intensive care admission, care and outcome among participants

Variable	ICU eligible n=36 (%)	HDU eligible n=16 (%)	t-test	X^2	P value	OR	95%CI
Condition at admission							
Pregnant	5(13.9)	0					
Postpartum	28(77.8)	16(100.0)		2.70	0.1582	1.57	1.26-1.96
Post abortion	3(8.3)	0		1.65	0.5411	0.00	0.00-4.53
GCS							
Mild	2(5.5)	9(56.3)					
Moderate	10(27.8)	5(31.2)		6.00	0.0401	0.111	0.01-0.93
Severe	24(66.7)	2(12.5)		4.42	0.0787	0.17	0.02-1.24
SPO_2							
Hypoxia	28(77.8)	15(93.8)					
Normal	8(22.2)	1(6.2)		1.974	0.2451	0.23	0.01-2.20
Respiratory rate							

Tachypnea	35(97.2)	14(87.5)					
Normal	1(2.8)	2(12.5)		1.926	0.2210	0.20	0.01-3.19
Pulse rate							
Normal	6(16.7)	7(43.7)					
Tachycardia	30(83.3)	9(56.3)		4.333	0.0791	0.26	0.06-1.15
Systolic BP							
<90	5(13.9)	0					
90-139	16(44.4)	10(62.5)		2.84	0.1472	1.63	1.20-2.20
≥140	15(41.7)	6(37.5)		0.51	0.4768	0.64	0.16-2.58
Organ dysfunction							
Yes	34(94.4)	11(68.8)					
No	2(5.6)	5(31.2)		6.278	0.0232	7.73	1.08-68.44
Organs involved							
1 organ	19(52.8)	8(50.0)					
≥2 organs	15(83.3)	3(18.8)		0.98	0.4824	0.47	0.08-2.51
Complication at ICU							
Yes	31(86.1)	5(31.2)					
No	5(13.9)	11(68.8)		15.307	<0.001	13.64	2.76-75.38
Mean duration of admission	3.47±2.613	3.56±3.966	-0.097		0.9232		
Mean total cost (naira)	48347±35370	34812±24296	1.388		0.1711		
Final outcome							
Alive							
Dead	12(33.3)	15(93.8)		16.20	<0.001	0.03	0.00-0.30
	24(66.7)	1(6.2)					

Discussion

In this study, 30.8% of the patients managed at the ICU were HDU eligible and would have been effectively managed at a HDU. This was similar to reports of Bhat et al., 2013 that 32.3% of ICU admissions were appropriate for HDU care and Zeeman, 2006 who concluded that a HDU care would have sufficed for 50% of obstetric patients requiring higher critical care. Thus, in the face of few ICUs in low resource countries, HDUs will reduce the burden on the ICUs as they have been shown to reduce ICU admissions as reported by Ryan et al., 2000. In addition, there was no statistical significant difference in the cost of care and duration of admission among ICU eligible and HDU eligible but managed in the ICU patients from this study. This further supports the conclusion of Zeeman, 2006 that a HDU provides opportunity for cost reduction in managing critically ill obstetric patients; bearing in mind that 68.8% of HDU eligible patients in this study were of low social class. The pattern of indications for admission into the ICU were similar between the two groups studied; thus, if established, the HDU will be

relevant as the indications or admission into them abound and PPH as the commonest indication for admission among HDU eligible participants was similar to the report of Saravanakumar et al., 2008. Among the HDU eligible women, 75% had no form of antenatal care and all were admitted for obstetric reasons. This was similar to the report from a HDU in India where 84.2% of the women had no antenatal care and 68.2% were admitted for obstetric reasons (Dattaray et al., 2013). It was not unusual that there were no statistical significance among ICU and HDU eligible women in terms of the systolic blood pressure, oxygen saturation, pulse rate and respiratory rate at admission into the ICU because these were effects of the need for systemic support which are the main criteria for critical care which are rendered at both ICU and HDU. The severity of the Glasgow coma score and multiple organ involvement may explain the statistical significance in the development of further complications after ICU admission in the two groups. This brings to the fore the distinction in the criteria for HDU care which emphasizes basic

respiratory support and single organ involvement. Thus, if these criteria are adhered to there should not be an increase in maternal mortality following the establishment of HDUs in low resource countries. The low mortality rate of 6.25% among HDU eligible patients in this study was lower than mortality rates of 12.28% from a HDU by Sarvanakumar et al., 2008.

There is no doubt that scarcity of resources for health care services in low resource countries remain a major challenge in these regions. An obstetric HDU can be a two bedded unit like reported by Ryan et al., 2000 in Dublin or 14 bedded as reported in a center by Saravanakumar et al., 2008; the size will depend on the quantification of the need, available manpower and resources in each locality. Furthermore, a survey of provision of HDU services by the Liverpool women's NHS Trust showed that majority of the HDUs were manned primarily by midwives of whom 77% of the personnel training were provided in-house (NHS 1996) with effective service delivery. Thus, in-house training could be employed with additional invited resource persons for personnel to man HDUs where release of personnel for overseas training may hinder availability of personnel in facilities in low resource countries.

Conclusion/ Recommendations

This study showed that about a third of patients admitted into ICU were HDU eligible with similar cost, duration of stay and lower mortality. We recommend the establishment of HDUs in low resource countries the size of which will depend on available resources with in-house training for

midwives who can primarily man the units with obstetrician and anesthetist supervision.

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Original Article

PRELIMINARY BIOAUTOGRAPHIC ANALYSIS OF THE SEEDS OF *GLYPHAEA BREVIS* (SPRENG) MONACHINO FOR ANTIOXIDANT AND ANTIBACTERIAL PRINCIPLES.

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ABTRACT

Glyphaea brevis (Spreng) Monachino (G. brevis) is widely distributed in West Africa with its various parts used in traditional medicine. In Sierra Leone, the leaves of the plant are used traditionally to enhance labour while the seeds are used in the treatment of skin infections. The present study is aimed at screening the seeds for phytochemical groups, antioxidant principles and antibacterial principles. The plant extracts were examined for phytochemical groups by standard spot tests. Chromatographic separation of seed extract was performed on reverse phase column and on silica at medium pressure chromatographic set-up. Thin layer chromatographic plates were visualised by UV light and vanillin sulphuric acid spray reagent. The crude extract and chromatographic fractions were examined for radical scavenging properties using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). Antibacterial bioautographic evaluation was performed using B. subtilis NCTC 8236 as test organism and Methylthiazolyl tetrazolium chloride as detecting reagent. Phytochemical screening of the seeds of G. brevis indicated the presence of phenolics and saponins. Pink colour reaction of polar Thin Layer Chromatographic (TLC) spots with vanillin-sulphuric acid spray (V/SA) suggested the presence of terpenoid glycosides. Screening with DPPH showed prominent antioxidant spots on silica at Rf 0.8, 0.5, 0.4 Ethyl acetate (EtOAc). The three spots reacted purple to V/SA. Bioautographic analysis revealed antibacterial spots at Rf 0.8(EtOAc) and 0.7(EtOAC-MeOH, 9:1).
The seeds of Glypheae brevis contain specific antioxidant and antibacterial principles which have been described by their chromatographic properties in this study.

Keywords: antioxidant, phytochemical, antibacterial,

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INTRODUCTION

Medicinal plants are being used in the treatment of diseases such as malaria, diabetes, sickle-cell anaemia, mental disorders (Elujoba, Odeleye & Ogunyemi, 2005) as well as microbial infections (Okigbo & Mmeka, 2006).

The use of traditional therapy is not restricted to developing countries alone; the Food and Agricultural Organisation reported in 2002 that at least 25% of drugs used in modern pharmacopoeia are derived from plants while many others are synthetic analogues based on prototype compounds isolated from plants. According to the World Health Organization (WHO, 2001), 80% of the worlds' population use medicinal plants in the treatment of diseases. This rate is much higher with an estimated 90% of the population in developing countries relying on the use of medicinal plants to help meet their primary health care needs (WHO, 2002).

In the developed countries, medicinal plants serve as the basis for drug development making traditional medicine an essential commodity in both the developed and developing countries. Medicinal uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoctions from the plants (Ogbulie, Ogueke, & Okorondu, 2004). *Glyphaea brevis* (Spreng) Monachino a Tilaceous shrub which produces fruits in duplets is widely distributed in West Africa. The various parts of the plant are used in traditional medicine across West Africa. In Sierra Leone, the leaves of the plant are used traditionally to hasten labour, and the leaves and stem bark are good fodder for goats. It is also used in the treatment of sleeping sickness, an aphrodisiac, as an antibacterial for an eye infection, and cleaning of the gum (Ogbonnia, Van-Staden, Jager & Coker, 2003). It is also known for its carminative effects and its use as an anticonvulsant in children.

The plant was first collected by Monachino, Joseph Vincent and is widely distributed in different countries across West Africa such as Liberia, Cote d'Ivoire, and Nigeria; filed as *Glyphaea brevis* (Spreng) Monachino, family Malvaceae. However unresolved controversy still surrounds the classification of the family Malvaceae. While the narrower circumscription of the family is monophylectic, the broader or *sensu lato* circumscription of Malvaceae is polyphylectic. In particular, the genus Glyphaea has been severally classified into the Malvaceae, Tilaceae, Gramicidaceae or Spermamiaceae (Heywood, Brummitt, Culham & Seberg, 2007).

The local name in Sierra Leone is Eh lontho ya Eh-Wor (Themne) or niki-guii (Gola), or Lamden (Kissi), or gbondo-kpokwe (Kono) and in bulontokawari (Limba). The Local name of *Glyphaea brevis* in Nigeria is Aloanyasi (Ibo) or Atori (Yoruba) (Ogbonnia, Van-Staden, Jager & Coker, 2003). In Senegal, it is called Tukulor Keki (Fula) or bola pane (Wolof). In Ghana, the local name is Foto (Akan) (Boateng, Bennet-Lartey, Opoku-Agyeman, Mensah & Fjeiser, 2004). The traditional uses of *G. brevis* are summarised in Table 1

The literature report on the chemistry of *G. brevis* is limited to the lipophilic contents of the leaves. Thus, a mixture of n-alkanes, Tetracosa (primary isolate), a mixture of fatty acid esters of primary alcohols, dotriancotanol and a mixture of Oleanolic and Echinocystic acids have been reported from the leaves of the plant (Mbosso, Ngouela, Ngueda, Beng, Rohmer & Tsamo, 2010).

When a free radical reacts with a non-radical, a free-radical chain reaction results and new radicals are formed. Attack of reactive radicals on membranes or lipoproteins starts lipid peroxidation. This can disrupt the natural balance and lead ultimately to oxidative stress (Halliwell, Gutteridge & Cross, 1992).

The external sources of oxidative stress can be drugs, carcinogens, hyperoxia, ozone, radiation, cigarette smoke and the cellular sources can be inflammatory cells, fibroblasts, xanthine, oxidase, NADPH, and endothelial cells.

Oxidative stress is known to contribute significantly to the process of inflammation, which underpins conditions like rheumatoid arthritis, inflammation, metabolic syndrome and diabetes, as well as to neurodegenerative diseases like Alzheimer's (Ding, Dimayuga, & Keller, 2007). Superoxide dismutase (SOD) along with catalase and glutathione peroxidase, form the front line of

the body's antioxidant enzyme defences. (McCord & Fridovich, 1969).

However, previous work have focus more on phytochemical screening and biological activity of the leaves and stem bark, with little or none on the seeds (Mbosso, Ngouela, Ngueda, Beng, Rohmer Tsamo, 2010). The present study is thus designed as a preliminary bioautographic screening of the seeds of *G. brevis* for anti-oxidant and antimicrobial principles.

Table 1: Traditional uses and Biological activities of *G. brevis*

Parts of plant	Ethno-medicinal uses	Chemistry	Biological activity
Leaves	Fodder for goats and sheep. Juice or Poultice to treat cuts and wounds (Nigeria) (Isidore, Akpan-Ebe, Olajide & Okon, 2011). Leaf enema is used to quicken labour in pregnant women, treatment of ulcers (Nigeria) (Gobah & Liang, 2011). Decoction for the treatment of sexually transmitted infections. Used as abortifacient, ecbolic, antiemetic, for eye treatment, liver problems and naso-pharyngeal affections, and as genital stimulants, pain-killers, treatment of pulmonary troubles, oedema and Gout (Nigeria) (Burkill, 1985)	Phenolic, Reducing Sugar, Phytosterols, Triterpenoids Alkaloids, Cardiac glycosides and Tannins detected. (Borokini & Omotayo, 2012)	Anticonvulsant effect in mice. Antioxidant effect. Antibacterial, anti-inflammatory (Ogbonnia, Van-Staden, Jager & Coker, 2003) (Dickson, Annan & Komlaga, 2011).
Roots	Decoction is used to treat stomach upset, indigestion and to increase appetite. Also used to treat diarrhea, dysentery, as febrifuge, for paralysis, epilepsy, convulsions and spasms (Cuendet, Hostettmann, Potterat & Dyatmiko, 1997).		.
Stem bark	Aphrodisiac, appetizer, laxative, and as a remedy for chest pains, diarrhea, dysentery, and sleeping sickness.	Phenolic, Reducing Sugar,Triterpenoid, Saponins and Flavonoids detected (Dakam, Oben & Ngogang, 2008).	Anti-inflammatory, Antioxidant and antibacterial effect (Dickson, Annan & Komlaga, 2011).
Seed	A Paste of the dry powder mixed with ashes for Ringworm Infection. (Boateng, Bennet-Lartey, Opoku-Agyeman, Mensah & Fjeiser, 2004).	-	-

MATERIALS AND METHODS

The sample of seeds, leaves and stem bark of *Glyphaea brevis* were collected on the 12th of May 2012 at Mayenkineh Road, Calaba Town in Freetown, Sierra Leone and authenticated at the Department of Pharmacognosy and Phytochemistry, University of Sierra Leone.

Reagents

Reagents used included, Vanillin sulphuric acid spray (V/SA) comprised of vanillin (1g) in concentrated sulphuric acid (100mL) that is used for detection of higher alcohols, phenols, terpenoids, steroids, essential oils and esters generally after heating at 120^0C for 1minute (Borokini & Omotayo, 2012).

Other reagents used were:2, 2- Diphenyl-1-picrylhydrazyl radical, DPPH, (1g) in methanol (100mL) which can detect antioxidant principles (radical scavenging) (Borokini & Omotayo, 2012); Dragendorffs Reagent, which is a mixture of solution A and B diluted to 100mL with H_2O; Solution A is comprised of Bismuth nitrate (0.17g) in AcOH (2mL) and H_20 (8mL) and added to Solution B: KI (4g) in AcOH (10mL) and H_2O (20mL); it is used to detect alkaloids. One other reagent used was ferric chloride ($FeCl_3$) solution: 1% iron (III) chloride solution is neutralised with sodium hydroxide until a slight precipitate of FeO(OH) is formed. The mixture is then filtered before use. It is used to detect the presence of phenol.

For the TLC, four solvent systems were used: Solvent A = EtOAc (Ethyl acetate), Solvent B = EtOAc/ MeOH ratio 9:1 (Ethyl acetate/Methanol),

Solvent C = MeOH- (Methanol), Solvent D= MeOH (50%)

An accelerated gradient chromatography (AGC) system was used comprising the set-up of AB-Separo (Sweden). This is a medium pressure chromatography under pressure with accelerated solvent flow to allow for the separation of bulk plant material into various fractions. It is used for preparative work.

Comparative Phytochemical Screening of Leaves, Stem Bark and Seeds of *G. brevis*Plant parts were air-dried for 14 days and macerated with methanol (20mL) for five days. The extract (1mL) of each plant part obtained after maceration with methanol was treated with a drop of ferric chloride solution and observed for any colour change (blue-black). The extract (1mL) was treated

with 2 drops of Dragendorff solution and observed for any reddish-brown precipitate, indicating presence of alkaloids. To each Plant part (200mg) was added distilled water (10mL) and the mixture shaken vigorously. This was observed for any frothing persisting longer than 30 minutes.
Different solvent compositions were tried on both the normal phase (Ethylacetate/ MeOH) and the Reverse phase plates (methanol/water) to resolve the components of *G. brevis.* The TLC plates were first observed under UV-light and then sprayed with Vanillin Sulphuric Acid (V/SA).

The powdered dried seed of *G. brevis* (580g) were extracted in cold methanol (1L) for 48 hours. The extract was clarified by filtration and evaporated to dryness *in-vacuo*.

Chromatography of Seed Extracts

Reverse-phase column chromatography of the bulk extract from the seeds of *G. brevis was carried out.* The crude extract (6g) was dissolved in MeOH (7ml) and loaded on Lobar reverse phase (C-18) glass column and then eluted in gradients of Water-Methanol. The process was repeated with the remaining crude extract (9g).
Fractions that reacted pink after reverse phase chromatography were pooled (1.5g) and subjected to AGC using Silica gel and gradients of n-hexane and ethyl acetate.

Bioautography for Antioxidant and

Antimicrobial Principles

Preparation of the TLC plate
Solutions of the test samples (GB) were spotted on reverse phase TLC plates and silica phase TLC and developed in solvent systems C, D and A, B respectively. The plates were then allowed to dry in air. The DPPH spraying reagent was used to spray the dried TLC plates and observed for any decolourisation over a period of 10 minutes.

Nutrient agar (14g) granules was weighed and suspended in distill water (500mL) in a flat bottom flask. The mouth of the flask was covered with foil

and the mixture was shaken together. This was heated to dissolve the particles completely. The clear solution was dispensed using a dispenser (15mL) into Mc Cartney bottles before allowing the mixture to cool. The bottles were tightly capped and placed in an autoclave for sterilization at 121^0 C for 15mins. The prepared nutrient agar was then inoculated with *Bacillus Subtilis NCTC 8236*.
Solution of the test samples were spotted on silica TLC plates in duplicate. The plates were then developed using solvent system B and then allowed to dry in air. A spreader was used to overlay the seeded agar on the TLC plates.
The spread agar was allowed to set on the plates and thereafter incubated at 37^0C for 18 hrs. After incubation, inhibition zones were made visible by spraying the plates with aqueous solution (2.5 mg/mL) of thiazolyl blue (Methylthiazolyltetrazolium chloride MTT) and incubated for 2h.

RESULTS

Seeds and Stem bark of *G. brevis* showed a blue black colour with ferric chloride in a test tube. The Leaves of *G. brevis* showed no change in colour with ferric Chloride. The seeds also showed reddish brown precipitate with Dragendorff reagent. No reddish-brown precipitate was observed for the Leaves and Stem bark when mixed with Dragendorff reagent. Frothing persisted for 24h when the seeds were shaken with water. No persistent frothing was observed for the leaves or the stem bark.

No mobile spot was detected on silica with Solvent Systems A (ethylacetate) or B (10% methanol in ethyl acetate) (NpTLC). However, the reverse phase TLC with methanol (RpTLC) yielded 0.8 UV and pink (V/SA reaction) for seeds; 0.8UV, pink (V/SA reaction) for stem bark and in 50% methanol the average yield for seeds was 0.19 UV and pink (V/SA) (Fig. 1).

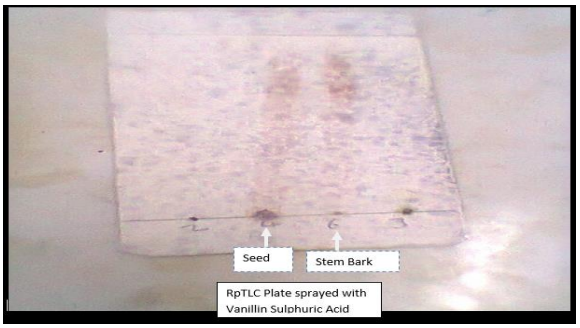

Figure 1: RpTLC Plate (solvent system D) sprayed with Vanillin Sulphuric Acid.

The combined total weight of the crude extract obtained after extraction and re-extraction is 18.9g which is 3.26 % of the total weight of the dried seeds of *G. brevis*. The crude extract of the seeds (GB) (6g) subjected to medium pressure chromatographic separation on reverse phase gave fractions, collected in test tubes (15mL). A Pinkish broad spot with vanillin sulphuric acid weighing 33mg was obtained in one of 15 tubes with 10% methanol solvent. A similar spot weighing 352mg was obtained with methanol. The remaining crude extract (9g) subjected to chromatographic separation on reverse phase as shown in Table 2. The combined fractions obtained from the reverse phase column chromatography, Fraction M, E and L were pooled and eluted on silica gel as shown in Table 3.

When the NpTLC plate (silica-solvent system B) of the extract of the seeds of *G. brevis* was sprayed with DPPH a reaction was observed at the origin as shown in Figure 2A. When the RpTLC plate (reverse phase-50% methanol) of the extract of the seeds of *G. brevis* was sprayed with DPPH a reaction was observed at a broad area around R_f 0.8 as shown in Figure 2B.

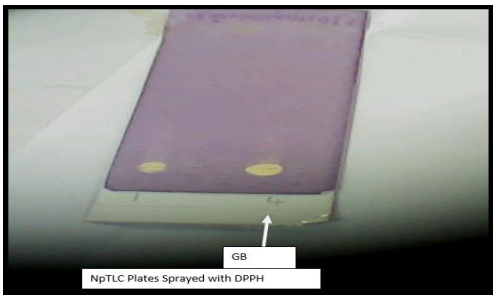

Figure 2A: GB on Normal Phase TLC (NpTLC) Plates Sprayed with DPPH

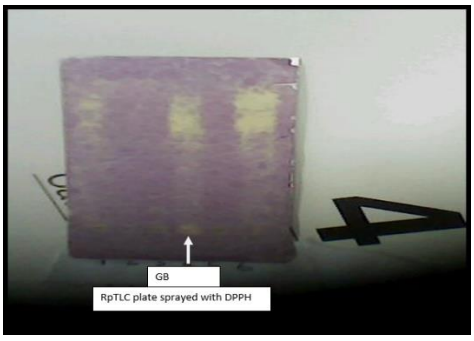

Figure 2B: GB on Reverse phase TLC Plate sprayed with DPPH

TABLE 2: Repeated Reverse Phase Column Chromatographic Separation

Solvent System/Volume	Collection of Fraction	Remarks
10% methanol(900ml)	Flask 1- 3	No detectable colour
20% methanol (500ml)	4- 10	Fraction 4- 11 were orange after spraying; spot turned ash after heating and became black after prolong heating (1.69g)
30% methanol	11- 12	Flask 12 - 19 showed pink colour observed only at the origin (Not DPPH reacting) The quantity was 366mg.
40% methanol	13- 14	--
50% methanol	15	--
60% methanol	16	--
70% methanol	17	--
100% methanol (washing)	18- 20	Flask 20 had weak antioxidant property, it's a mixture of 5 pink spots (Fraction E-999mg). (V/SA) Flask 18 had 3 pink spots (Fraction L-156mg-).

TABLE 3: Silica Gel-Chromatography Separation of Fractions Obtained from Reverse-Phase Chromatography

Solvent system	Tube Number
n-hexane (100%)	1-3
n-hexane/EtOAc (50%)	4-6
n-hexane/EtOAc (30%)	7-10
EtOAc(100%)	11-15
EtOAc/methanol (90%)	16-20
EtOAc/methanol (80%)	21-25
EtOAc/methanol (70%)	26-30
EtOAc/methanol (60%)	31-36
EtOAc/methanol (50%)	37-41
methanol (100%)	42-43

The various column fractions of the seed extract were re-examined for homogeneity by TLC (V/SA) and antioxidant properties (DPPH), as shown in Table 4.

Antimicrobial Bioautography

Samples tested for antimicrobial activity, by bioautography, are the crude extract GB and the final silica column fractions GB 5, GB 21 and GB 43. Antibacterial activities were seen as a very whitish spot against a purplish background. GB 5 showed antibacterial activity at R_f 0.9. The antibacterial activity of the spot was observed at corresponding R_f 0.9 in the crude extract (GB). GB 21 was active against the tested organism (*B. substilis*) with a very clear whitish spot against a purplish background as shown in Figure 3. GB 21 showed an antibacterial spot at R_f .0.7. The duplicate plate

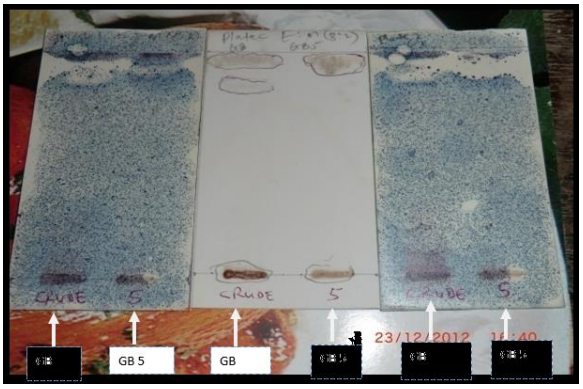

Figure 3: Antibacterial Activity (*Bacillus Subtilis NCTC 8236***) of Crude Extract GB and GB 5**

sprayed with V/SA, however, indicated three spots at R_f 0.7, 0.8, 0.9.

DISCUSSION

The Crude extract (GB) of the seeds of *G. brevis* gave a reddish brown precipitate in Dragendorffs reagent indicating the presence of alkaloids. Ferric chloride test gave a blue-black colour indicating the presence of phenolics. Frothing was also observed with crude extract suggesting the presence of saponins.

The leaves of *G. brevis* did not show any reddish brown precipitate with Dragendorff's reagent. Ferric chloride test did not show blue-black colour and no frothing was observed. Thus, indicating the absence of alkaloids, phenolics and saponins.

The Stem bark of *G.brevis* showed a blue-black colour with ferric chloride test indicating the presence of phenolics. No reddish brown precipitate was observed for Dragendorff reagent and no frothing was observed indicating the absence of alkaloids and saponins.

Preliminary TLC studies of the different parts of *G. brevis* on silica with mobile phase as ethylacetate or 10% methanol in ethylacetate did not show mobility of components beyond the origin. However, reverse-phase TLC with 50% methanol revealed pink-reacting spots (V/SA spray) for both the seed extract (Rf 0.8, broad) and the stem bark extract (Rf 0.7 and 0.8, both broad). This is suggestive of the presence of polar terpenoid compounds (terpene glycosides) in both the seeds and the stem bark. The chemistry of the stem bark by Dakam, Oben & Ngogang in 2008 showed the presence of Phenolics, Triterpenoids and Saponins.

100% methanol showed a better resolution of the seed extract, indicating pink-reacting spots at R_f 0.36, 0.13, and 0.07.

As a follow-up to the preliminary TLC studies attempts were made to isolate pure components of the seed extract by repeated medium-pressure chromatography (reverse-phase and normal-phase) as discussed subsequently. Reverse phase column chromatography eluted a 33mg fraction with MeOH (10%) that reacted pink with vanillin Sulphuric acid as shown in Table 4. This 33mg sample gave a pink spot (lipophilic, non-uv detectable). Fractions from tubes 15 to 23 reacted light brown and became ash after heating (Supposed sugar present). Methanol as solvent system eluted fraction M (352mg) that also reacted pink with vanillin Sulphuric acid.

On further reverse phase column chromatography separation of the remaining crude extract (9g) with Methanol, fraction L (999mg) and fraction E (156mg) was obtained. Fraction E showed five pink spots, whilst fraction L showed three pink spots. Fraction E and L was also found to be weakly antioxidant. This indicated that the three fractions M, E and L are not pure fractions but mixtures.

The three Fractions M, E, and L were pooled (1.5g) and subjected to silica gel chromatography as shown in Table 4 showing evidence of the presence of antioxidants in G.*brevis*.

The crude extract (GB) of *G. brevis* was poorly resolved on NpTLC (showing decolourisation at the origin). On RpTLC the crude extract (GB) showed reaction with DPPH at R_f 0.8 (solvent D). This is also consistent with the reaction of eluted fractions of *G. brevis* as shown in Table 7. Thus, GB 1, GB 5, GB 6, GB 9, GB 26, and GB 43 all showed immediate reaction with DPPH, on silica. The prominent antioxidant spots in the fractions were observable at R_f 0.8, 0.5, 0.4 (solvent A); 0.2 (solvent B) and 0.14 (solvent C). This infers the presence of antioxidant

Table 4: Bulking and TLC Profile of the Various Fractions of *G. brevis*

Bulked Fractions	Mobile phase, Rf, (UV detection)) (V/SA colour detection),	DPPH reacting spots*	Quantity
Fractions 1- 4 (GB 1)	A; 0.8 (Purple)	Immediate	68mg
Fraction 5(GB 5)	A; Two spots 0.8 (uv detectable) (purple) ; 0.5 (non-uv detectable), (Purple)	Immediate	125mg
Fractions 6-8(GB 6)	A; two spots; 0.8 (uv detectable; 0.5 (non-uv detectable), (Purple)	0.5 Immediate	317mg
Fractions 9-13(GB9)	A; 0.4 (non-uv detectable) (purple) (only slightly impure)	0.4 (immediate)	35mg
Fractions 15-17 (GB 15)	A; 0.2 (single pure spot), (Purple)	Weakly antioxidant	12mg
Fractions 21- 25 (GB 21)	B; Three spots; 0.7; 0.8; 0.9	-Weakly antioxidant	45mg
Fractions 26-27 (GB 26)	B; Two spots; 0.4; 0.2(Purple)	0.2 (immediate)	17mg

principles in the seeds of *G. brevis*(Borokini & Omotayo, 2012)..

The crude extract (GB) of *G. brevis* showed antibacterial activity when tested with *B. substilis*. This activity was replicated by GB 5 at R_f value about 0.9 (solvent A), as shown in figure 4.



Figure 4: Antibacterial Activity (*Bacillus Subtilis NCTC 8236*) of G.B 21

GB 21 and GB 43 were also tested for antibacterial activity. GB 21 showed antibacterial activity with spot at about R_f 0.7 (solvent B). However, a duplicate TLC plate sprayed with V/SA showed that GB 21 is a mixture with three spots at Rf 0.7, 0.8 and 0.9. GB 43 had no antibacterial activity but showed prominent antioxidant activity. Work done on *G. brevis* by Ogbonnia, Van-Staden, Jager & Coker in 2003 and Dickson, Annan & Komlaga in 2011 also reported biological activity that includes antibacterial and antioxidant activity.

CONCLUSION

Phytochemical screening of the seeds of *G. brevis* indicated the presence of phenolics and surface-active agents presumed to be saponins. No alkaloid reacting spot was detected on TLC in spite positive colour reaction of the crude extract in the spot test. False alkaloid reactions to Dragendoff's reagent are known. Pink colour reaction of polar TLC spots with vanillin-sulphuric acid spray (V/SA) suggested the presence of terpenoid glycosides.

Screening of the various column fractions with DPPH showed three prominent antioxidant spots on silica at R_f 0.8, 0.5 and 0.4 (solvent system A). The three spots reacted purple to V/SA. Another purple reacting antioxidant spot was observable at R_f 0.2 (10% methanol in ethylacetate - solvent system B). The fifth prominent antioxidant spot reacted pink to V/SA at R_f 0.14 (solvent system C).

Bioautographic analysis revealed two antibacterial spots on silica at Rf 0.8 (solvent system A) and 0.7 (solvent system B).

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