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INFORMATION:

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INFORMATION

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QUALITY OF ANTIBIOTICS PRESCRIBED IN SELECTED OUTPATIENTS HEALTHCARE FACILITIES IN PAPUA NEW GUINEA

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ABSTRACT:
The study was conducted to evaluate compliance of antibiotics prescribing in three selected outpatients healthcare facilities in Papua New Guinea (PNG), to the country specific Standard Treatment Guidelines (STG) and to identify factors influencing prescribing pattern. The study was carried out in the Losuia Health Centre (LHC), Alotau Provincial Hospital and Port Moresby General Hospital (PMGH) outpatient departments. The study sample involved 300 participants at each setting. Oral amoxicillin products, chloramphenicol and co-trimoxazole made up approximately 70% of the antibiotics prescribed to 637/1090 of patients. Almost one-quarter (24.4%) of prescriptions for antibiotics were non-compliant selections. At the LHC approximately 20% of both dosage and duration errors occurred. Overall non-compliant prescribing for children was approximately 50% but significantly more compliant at PMGH (P= 0.0058) contrasting with the other settings. At the LHC only 30.6% of antibiotic prescriptions for children were compliant with STG requirements and fulfilled PNG regulatory requirements. With respect to the STGs, Community Health Workers (68.0%) and Nurse Officers made more non-compliant antibiotic selections. High levels of antibiotic prescribing combined with high levels of non-compliant antibiotic prescribing as compared to PNG-STGs, were identified in this study. This is a disturbing finding as it raises many questions related to quality assurance of health care interventions in PNG. The data also raises a clinical concern for the high level of oral chloramphenicol prescribed in out-patient settings.

Keywords: Antibiotics, Resistance, Prescribing, Developing Country, Compliance, Standard Treatment Guidelines

INTRODUCTION:

Globally, antibiotics are one of the most frequently prescribed drug groups and there are reported concerns about the continuous indiscriminate and excessive use of antimicrobial agents including antibiotics, that promote the emergence of resistant organisms [1, 2]. Appropriate antibiotic prescribing is the first step for optimum antibiotic use and has the potential impact of reducing resistant micro-

organisms [3]. In Papua New Guinea (PNG), the prescribing of antibiotics is performed by non-medical staff including Community Health Workers (CHWs), Nursing officers (NOs) and Health Extension Officers (HEOs) in health centres and mainly by medically trained staff at district and referral hospitals. Irregularly updated Standard Treatment Guidelines (STGs) have been available to these prescriber groups for decades [4]. In PNG, healthcare is primarily provided by the public sector with limited private facilities available in larger towns. Aid-posts provide basic care and health centres provide out-patient and limited in-patient care. Recently, the World Health Organization (WHO) released a report detailing the prevalence of antimicrobial resistance worldwide [5]. This indicated that antibacterial drugs have been prescribed/ used worldwide for several decades including extensive misuse. In the WHO Western Pacific region which includes Papua New Guinea (PNG), no specific resistance data were reported by the WHO for pathogens in PNG [5].

Resistance to selected antibiotics has been reported in PNG over the past 15 years. For example, Duke in 2000, found high levels of resistance to commonly prescribed antibiotics [6]. The report also indicated that a notable factor causing child mortality in PNG was the severity of sepsis arising from Gram-negative bacteria which were resistant to standard antibiotics [6]. Manning et al. in 2011 reported that for children with acute bacterial meningitis, all of the Haemophilus influenzae isolates were resistant to chloramphenicol [7]. An outbreak of nosocomial infection of Klebsiella pneumonia in hospitalised neonates in PNG showed resistance to cefalosporins which increased from 25% to 73% over 13 months with a patient mortality rate of 40% [8].

A recent study of outpatient antibiotic use in children based on the Integrated Management of Childhood Illness (IMCI) guidelines showed 54% of cases were treated with antibiotics and 40% of these were treated in a manner not compliant with the STGs. In addition, 29% received antibiotics when they were not recommended and 11% did not receive them when they should have [9].

In addition to reported high levels of antibiotic resistance and inappropriate use in PNG, anti-infectives including amoxicillin tablets, although not expired, were found upon laboratory analysis to contain less than their minimum specified active ingredient [10]. Furthermore, when unexpired antibiotic collected from community pharmacies in PNG were analysed, one batch of amoxicillin tablets was sub-standard and another counterfeit [11].

Purchasing and ensuring the ready availability of antibiotics of reliable quality, followed by optimum antibiotic prescribing according to STGs, can reduce the prevalence of resistant micro-organisms generated by excessive use [12]. Inappropriate prescribing of antibiotics has been identified in many health facilities in other developing countries [13-15]. High levels of

antibiotic prescribing were also linked to specific health problems. For example, in Bangkok, Thailand, 63% of patients with upper respiratory tract infections were prescribed an antibiotic. Although patients were more likely to be prescribed antibiotics for bacterial conditions, 60% of those with likely viral conditions were prescribed antibiotics [16, 17]. In Indonesia, 90% of the 273 cases of fever of unknown origin were prescribed antibiotics, despite antibiotics not being recommended for this diagnosis [18].

This study aimed to evaluate prospectively, compliance of antibiotics prescribing in three selected outpatient healthcare facilities in PNG, to the STGs and to identify factors influencing prescribing quality in these settings.

METHODOLOGY:

A prospective study was carried out at Losuia Health Centre (LHC), Alotau Provincial Hospital (APH) and Port Moresby General Hospital (PMGH) in PNG. All outpatients with written antibiotic prescription orders including the diagnosis written by the prescriber were obtained from each patient's health book and, where necessary, from patient interviews. The health workers at each setting were informed of the study but not its specific objectives. The study was approved by the Human Research Ethics Committee of Curtin University (PH-10-2010), the Milne Bay Province Medical Research Advisory Committee for LHC and APH and the PMGH Executive Committee for PMGH. Only patients who gave consent to participate in the study were included.

At LHC, the interviews were undertaken by a CHW who spoke to the patients using their local dialect and the researcher recorded the answers. At APH, the interview was undertaken by the researcher with assistance from the pharmacist and pharmacy technician. At PMGH, patient interviews were carried out at the hospital pharmacy when patients came to collect their medicines. The interview was undertaken by the researcher with assistance from the pharmacists and pharmacy technicians.

Data recording:

A data recording sheet was developed and used to collect data for each antibiotic prescribed in the health facilities. The data recording sheet collected the participant's prescription details and included: patient's date of birth/age, weight, gender, and date attending clinic, name of healthcare location, current diagnosis, all current medicines prescribed (name, dose, frequency, duration, total number supplied), prescriber category, injections given including total number supplied. The quantity and antibiotic supplied and the prescriber level restrictions were recorded. Data on current and relevant previous diagnoses and medications prescribed were recorded from a health booklet which was kept by each patient. A participant of thirteen (13) years or less was classified as a child.

The Pidgin-English language is the language widely spoken in PNG and was used for oral communication throughout the study while English was used as appropriate.

At LHC, patients were referred to see either a NO, or a CHW. The complicated cases were referred to the HEO. If the case was serious, the patient was referred to APH for further investigation. This hospital was not easily accessible as it involved 10-12 hours of travel by boat or 30 minutes by air subject to scheduling and funds. Either service was only available approximately once a week. At APH and PMGH, patients were referred to see either a NO, HEO or Medical Officer (MO). The complicated cases were referred to the Specialist Medical Officer (SMO) or the Senior Specialist Medical Officer (SSMO). The diagnosis and prescribed medicines were written into the patient's health book by the prescriber, which the patient kept. The patient then proceeded to the Pharmacy for the supply of medicines. After receiving their medicines, the patient was then asked to see the researcher where consent was sought.

Selection of subjects:

The study sites were chosen for convenience to appropriately represent the tiered levels of health care delivery in PNG. Consecutive outpatients seeking medicines during the two-week study period at each site were screened and if eligible, were invited to participate in the study. Subjects were deemed eligible provided they carried with them their health book and consented to participate in the study. However, eligible subjects were excluded if they were too ill to be interviewed, could not communicate or there was insufficient time for the consultation. In the case of children, their parent or guardian was interviewed.

Analysis of data:

Compliance of each antibiotic prescription with the relevant PNG STGs current at the time of the study was assessed. The STGs consisted of the Manual of Family Planning for Doctors, HEOs and Nurses in PNG [20]; Standard Management of Sexually Transmitted Infections and Genital Conditions [21]; Standard Treatment Guidelines for Children in PNG [22]; and, Standard Treatment for Common Illnesses of Adults in PNG [23]. The criteria were based on the antibiotic selection, dosage, frequency and duration as specified in the STGs in respect to the diagnosis written by the prescriber. Furthermore, "Overall compliance" was defined as compliance with the previous four criteria plus the category of the prescription item complying with legislative prescriber level restrictions for non-medical prescribers as defined in the Medical and Dental Catalogue (MDC) [24] and for the quantity of each antibiotic dispensed (issued to the patient or guardian). The diagnosis used was that recorded by the prescriber in the patient's health book which was viewed at the time of the interview. The antibiotic prescribed and the number dispensed were checked against the prescribed duration stated in the STGs.

Allocation of prescribing compliance was made by the researcher where no doubt existed and this was reviewed by two experts. Where doubt regarding the allocation occurred, a consensus decision was made by the researcher and the two independent experts. Where the antibiotic selected was deemed inappropriate with the STGs, dosage, frequency and duration were not determined. Data from the recording sheets were entered into an Excel® spread sheet. Prescribed antibiotics were classified according to the ATC codes [25]. Disease states were classified according to the diseases stated in the STGs with reference to the Australian Medicines Handbook [26].

Simple descriptive statistics were used to summarise the data with respect to prescribing patterns, and the numbers and proportions of prescriptions which were classified as compliant within each age group and setting. A Generalised Estimating Equation (GEE) was used to identify any significant differences in compliance between the different compliance criteria, and between settings and age-groups. This particular model was used instead of the logistic regression model, because it took into account the correlations between observations on the same participant. Results of the analysis are reported similarly to a logistic regression, with odds ratios relative to some reference category, their 95% confidence intervals, and p-values. Analyses were performed using the statistical software program SAS version 9.2 (SAS Institute Inc, Cary, USA, 2008), and a p-value<0.05 was taken to indicate a statistically significant association in all tests.

RESULTS:

The data reported in this paper have been derived from a separate and expanded analysis of overall prescribing investigated at the three out-patient locations [19]. Antibiotics included in this study were: antibacterials (penicillins, other antibacterials, cefalosporins and other reserved antibiotics) listed in the MDC [24] with reference to the Australian Medicines Handbook [26] and excluding antituberculosis, antileprosy drugs, antifungals, antivirals, antiretrovirals, antiprotozoals, antimalarials and anthelmintics. The scope was also informed from the WHO ATC lists of antibiotics [25]. The overall prescribing parameters for antibiotics at the three locations, relative to overall prescribing, are summarised in Table 1.

Table 1 Antibiotic prescription data according to patient, drug and age group at each outpatient location and their proportion of all drug prescribing; P-values are obtained from Chi-square tests, unless otherwise specified.

Variable	Subgroup	LHC (%) N=356	APH (%) N=318	PMGH (%) N=416	Total N =1090	P-value
Antibiotics prescribed(patient based)		219/356 (61.5)	185/318 (58.2)	233/416 (56.0)	637/1090 (58.4)	0.2999
Antibiotics prescribed (drug based)		279/828 (33.7)	202/696 (29.0)	278/971 (28.6)	759/2495 (30.4)	0.0496*
Antibiotic injections+ (patient based)		42/219 (19.2)	5/185 (2.7)	11/233 (4.7)	58/637 (9.1)	<0.0001
Gender (frequency of antibiotic prescribing)	Female	106/192 (55.2)	89/178 (50.0)	112/202 (55.4)	307/572 (53.7)	0.4958
	Male	113/164 (68.9)	96/140 (68.6)	121/214 (56.5)	330/518 (63.7)	0.0174
Age group (frequency of antibiotic prescribing)	Adult	120/216 (55.6)	103/212 (48.6)	164/320 (51.2)	387/748 (51.7)	0.3438
	Child	99/139 (71.2)	82/106 (77.4)	69/96 (71.9)	250/341 (73.3)	0.5224

*p-value calculated using a GEE model. +Based on numbers of patients prescribed antibiotics
The p-values compare the distributions of these demographic variables across locations. Age group was missing for one record at LHC

The proportions of females who were prescribed antibiotics were similar across locations, but it appeared that a smaller proportion of males at PMGH received them compared to the other sites. Overall, a significantly greater proportion of males received antibiotics compared to females (330/578 [63.7%] vs 307/572 [53.7%]; p=0.0008). While there was no significant difference across sites in the prescriptions for adults or children separately, it appeared that, overall, a significantly higher proportion of children received antibiotics than adults (250/341 [73.3%] v 387/748 [51.7%]; p<0.0001).

Over 70% of children were prescribed an antibiotic at each consultation. Approximately 10% of all antibiotic prescriptions included more than one antibiotic. Patients not prescribed any drugs were not included in the study.

Table 2 Disease classifications for patients prescribed antibiotics

Diseases^	Frequency*	(%)
15.4 Acute soft tissue injuries	126	16.30
5.5 Malaria	116	15.00
19Asthma, COPD	110	14.23
19.5Cough	75	9.70
5.1 Bacterial infections	38	4.92
12.1 Dyspepsia, reflux & peptic ulcers	38	4.92
19.1 Bronchiolitis	38	4.92
Others	232	30.01
Total	773	100.00

*Subjects may have more than one diagnosis; ^Disease classifications based on the STGs with reference to the Australian Medicines Handbook classification [26]

The most common disease classifications for patients who were prescribed antibiotics at all locations are reported in Table 2. Possibly, owing to a lack of resources for health care staff to be able to differentiate the cause of a fever, or because patients were living in remote areas where antimalarials and antibiotics were prescribed together on many occasions. The match between the diagnosis and antibiotic prescribing varied widely with location. For example, at APH every upper respiratory tract infection diagnosis was prescribed amoxicillin, at PMGH scabies treatment included oral antibiotics and simple cough/fever was routinely managed with antibiotics.

Most prescribed antibiotics during the period of this study were amoxicillin oral products, followed by chloramphenicol, which was consistently prescribed at all locations (Table 3). Notably, three antibiotics made up approximately 70% of all antibiotics prescribed. Most antibiotics were for oral administration, with some antibiotic injections especially at LHC; very few antibiotic ear or eye drops or ointments were prescribed. The compliance of antibiotic prescribing overall and at each location is reported in Table 4 based upon each prescribing parameter assessed.

Table 3 Number of antibiotic items grouped according to antibiotic class prescribed at each location

Antibiotics	LHC (%)	APH (%)	PMGH (%)	Total (%)	P-value
Amoxicillin products	104 (37.3)	104 (51.5)	147 (52.9)	355 (46.8)	<0.0001
Chloramphenicol	40 (14.3)	25 (12.4)	30 (10.8)	95 (12.5)	
Co-trimoxazole	49 (17.6)	24 (11.9)	22 (7.9)	95 (12.5)	
Metronidazole	32 (11.5)	11 (5.5)	17 (6.1)	60 (7.9)	
Penicillin	35 (12.5)	7 (3.5)	11 (4.0)	53 (7.0)	
Cloxacillin	8 (2.9)	18 (8.9)	23 (8.3)	49 (6.5)	
Doxycycline	5 (1.8)	2 (1.0)	9 (3.2)	16 (2.1)	
Erythromycin	1 (0.4)	7 (3.5)	6 (2.2)	14 (1.8)	
Others	5 (1.8)	4 (2.0)	13 (4.8)	22 (2.8)	
Total	279 (36.8)	202 (26.6)	278 (36.6)	759 (100.0)	

Table 4: Compliance of prescribed antibiotics at Alotau Provincial Hospital (APH) Losuia Health Centre (LHC) and Port Moresby General Hospital (PMGH) including each indicator assessed and relevant interactions

Variable	Compliant n/N (%)	Odds Ratio	95% CI	p-value
(A) Compliance				
Dosage	516/574 (89.9)	17.20	7.51 - 39.38	<0.0001
Duration	521/574 (90.8)	13.46	6.25 - 28.98	<0.0001
Selection	574/759 (75.6)	1		
Dosage APH	131/136 (96.3)	0.43	0.15 - 1.27	0.1261
Dosage LHC	185/235 (78.7)	0.07	0.03 - 0.18	<0.0001

Dosage PMGH	200/203 (98.5)	1		
Duration APH	130/136 (95.6)	0.47	0.17 - 1.25	0.1296
Duration LHC	192/235 (81.7)	0.11	0.05 - 0.25	<0.0001
Duration PMGH	199/203 (98.0)	1		
Selection APH	136/202 (67.3)	0.77	0.51 - 1.16	0.2068
Selection LHC	235/279 (84.2)	1.98	1.27 - 3.09	0.0028
Selection PMGH	203/278 (73.0)	1		
(B) Compliant Selection				
Location				
APH	136/202 (67.3)	0.21	0.09 - 0.48	0.0002
LHC	235/279 (84.2)	1.08	0.43 - 2.72	0.8664
PMGH	203/278 (73.0)	1		
Location & Age				
APH Adult	82/115 (71.3)	1.56	0.84 - 2.90	0.1563
APH Child	54/87 (62.1)	1		
LHC Adult	128/158 (81.0)	0.48	0.23 - 1.01	0.0535
LHC Child	107/121 (88.4)	1		
PMGH Adult	135/201 (67.2)	0.25	0.12 - 0.53	0.0003
PMGH Child	68/77 (88.3)	1		
(C) Compliant Dosage				
Location				
APH	131/136 (96.3)	0.44	0.10 - 1.92	0.2737
LHC	185/235 (78.7)	0.06	0.02 - 0.20	<0.0001
PMGH	200/203 (98.5)	1		
Age group				
Adult	335/345 (97.1)	8.68	4.17 - 18.04	<0.0001
Child	181/229 (79.0)	1		
(D) Compliant Duration				
Location				
APH	130/136 (95.6)	0.43	0.12 - 1.56	0.1973
LHC	192/235 (81.7)	0.09	0.03 - 0.26	<0.0001
PMGH	199/203 (98.0)	1		
Age group				
Adult	314/345 (91.0)	0.87	0.47 - 1.63	0.6627
Child	207/229 (90.4)	1		
(E) Compliant on all criteria concurrently				
Location				
APH	125/202 (61.9)	0.27	0.13 - 0.55	0.0003
LHC	155/279 (55.6)	0.21	0.10 - 0.40	<0.0001
PMGH	195/278 (70.1)	1		
Location & Age				
APH Adult	77/115 (67.0)	1.68	0.92 - 3.05	0.0894
APH Child	48/87 (55.2)	1		
LHC Adult	98/158 (62.0)	1.66	1.01 - 2.75	0.0470
LHC Child	57/121 (47.1)	1		
PMGH Adult	132/201 (65.7)	0.40	0.21 - 0.77	0.0058
PMGH Child	63/77 (81.8)	1		
(F) Compliant on all criteria, plus satisfying prescriber rules				
Location				
APH	116/202 (57.4)	0.36	0.18 - 0.70	0.0029
LHC	109/279 (39.1)	0.16	0.08 - 0.30	<0.0001
PMGH	175/278 (63.0)	1		
Location & Age				
APH Adult	72/115 (62.6)	1.66	0.92 - 2.99	0.0941

APH Child	44/87 (50.6)	1		
LHC Adult	72/158 (45.6)	1.67	0.98 - 2.83	0.0584
LHC Child	37/121 (30.6)	1		
PMGH Adult	118/201 (58.7)	0.48	0.27 - 0.88	0.0180
PMGH Child	57/77 (74.0)	1		

The frequency of administration was also assessed but the data were not included in Table 4 since overall 98.6 % of prescription items had a compliant frequency, the lowest being 97.0% at LHC.

Overall almost one quarter of antibiotic prescription items were non-compliant to antibiotic selections (Table 4A). These included 44 (23.8%) when no antibiotics should have been selected for the diagnosis and 46 (24.6%) where a specific antibiotic was indicated in the guidelines but a different one was selected. It is notable that prescribing of compliant dosages and durations were significantly better than antibiotic selections. Considering the interaction of location with these parameters (Table 4A), using PMGH as the reference, LHC had significantly more compliant selections. LHC data highlighted different prescribing issues with significantly lower compliant dosages and durations of treatment, when compared with PMGH. The analysis in section (A) of Table 4 is based on 1907 records (one record for each type of compliance: (759+574+574) while the other sections of the table are based on either 759 records or the 574 records where drug selection was compliant.

It is noted that, when selection ("Compliant Selection" Table 4B) is based on the 759 antibiotic prescription records, and after adjustment for age-group, APH stands out as significantly less compliant (P = 0.002) compared to LHC and PMGH. After adjustment for differences in compliant selections between location, compliant selections for children were significantly higher (p = 0.0003) than for adults at PMGH. With respect to compliant dosage (Table 4C) overall adults received significantly (8.68 fold; P < 0.0001) more compliant dosing and LHC was significantly less compliant P < 0.0001) than PMGH. APH was not significantly different from PMGH with regard to dosing (96.3% compliant vs 98.5%). It is notable that, after adjustment for differences between settings, dosages for children were significantly less compliant than for adults (p < 0.0001).

In terms of compliant duration times of antibiotic treatments (Table 4D) it is evident that LHC prescribed significantly less compliant antibiotic durations than the other settings, however overall durations for adults and children were similar.

When considering compliance when all three criteria were combined (Table 4E), so that non-compliance on any one (or more) criteria rendered prescribing of the antibiotic non-compliant, both APH (P = 0.0003) and LHC (P < 0.0001) performed significantly poorer than PMGH in overall prescribing compliance. It is notable that only 155/279 of antibiotics

prescribed at LHC were compliant overall. Evaluating the interaction of setting and age identified that only 57/121 of antibiotic prescriptions for children at LHC were compliant. There was an interaction between location and age-group where antibiotic prescribing for children was significantly more compliant than for adults at PMGH whereas the opposite appeared to be true at LHC and no significant difference in age groups occurred at APH.

The non-compliance of antibiotic prescribing when additional factors (prescriber level restrictions and number dispensed) were assessed at each location is also shown in Table 4F. These other factors were whether the non-medical prescriber was authorised to prescribe particular medicines according to the prescriber restrictions defined in the MDC [24], and whether the antibiotic and/or quantity dispensed were correct. This latter criterion included no supply (stock not available), or an undersupply or oversupply according to the dosage and duration prescribed; including these regulatory requirements identified, only 109/279 of antibiotics prescribed at LHC met all STG prescribing and PNG regulatory requirements. When based on PMGH it performed 6.25 times more poorly in meeting these requirements. It is notable that the interaction of setting and age found only 37/121 of antibiotic prescriptions at LHC met all STG and PNG regulatory requirements. It is also evident that PMGH provided significantly more compliant antibiotic prescriptions for children than LHC ($P < 0.001$) or at APH ($P= 0.0029$).

Data in Table 5 shows the level of compliant prescribing with respect to prescriber category. Medically qualified prescribers made more non-compliant drug selections with respect to the STGs but their dosages, frequencies and treatment durations closely followed the STGs. Non-medical prescribers made more non-compliant drug dosage and duration errors.

Table 5: Overall compliance of prescribing antibiotics by prescriber category. P-value from Chi-square

Prescribers	Non-compliant (%)	Compliant (%)	Total (%)	P-value
CHW	53 (68.0)	25 (32.0)	78 (10.3)	<0.0001
DT/RDO/DO	1 (7.7)	12 (92.3)	13 (1.7)	
RHEO/HEO	61 (49.6)	62 (50.4)	123 (16.2)	
RMO/MO/SMO	154 (39.3)	238 (60.7)	392 (51.6)	
NO	85 (55.6)	68 (44.4)	153 (20.2)	
Total	354 (46.6)	405 (53.4)	759 (100.0)	

Legend: Inapp = Inappropriate; App = Appropriate; CHW = Community Health Worker; DT = Dental Therapist; RDO = Resident Dental Officer; DO = Dental Officer; NO = Nursing Officer; RHEO = Resident Health Extension Officer; HEO = Health Extension Officer; RMO = Resident Medical Officer; MO = Medical Officer; SMO = Senior/Specialist Medical Officer

DISCUSSION:

This study reports specific data in PNG on the compliance of antibiotic prescribing for adults and children from outpatient healthcare facilities, related to official health department guidelines. High levels of non-compliant prescribing occurred with all prescriber groups with the exception of dentists (but they were responsible for very few prescriptions). Most prescribing was performed by nurse officers and medical doctors. Overall in this study, 58.4% (637/1090) of patients who were prescribed drugs received an antibiotic independent of location. This percentage is higher than the 54% reported in a cohort of children outpatients in a recent PNG study [9]. It is also higher than the WHO/INRUD prescribing indicators, which from the period 1982-2006 was just below 50% [27]. Prescribed antibiotics constituted 759/2495 of all drugs prescribed over all the studied locations. Although the prescribing of antibiotics in this study was high, it was also noted that at LHC, there were overall few patients diagnosed with chronic diseases. The diagnoses were usually for acute conditions. However, many more patients with chronic diseases were included in the total cohort at PMGH with only a small non-significant decrease in antibiotic prescribing. It is possible that high antibiotic prescribing could be influenced by poor accessibility since many families are obligated to walk for several hours, even up to a day, to attend their health care facilities. This makes it difficult for them to easily return for another consultation if their condition deteriorates. It is unknown whether some were prescribed as a precautionary supply since no evidence to support this possibility was available from the patient's health booklet.

Studies in other developing countries have shown similar results. In Yemen [28], antibiotics were prescribed in 51.0% of patient encounters and 23.8% of the total number of prescribed drugs in public hospital outpatients. A higher percentage (65.0%) of antibiotic use was recorded in the health care facilities in Ghana [29]. A pilot study carried out in three health centres in Cambodia, showed that the percentage of antibiotics prescribed ranged from 10.0% to 66.0% [30]. A Jordanian Hospital outpatient department found that the average percentage of prescriptions involving antibiotics was 35.6% from 187,822 prescriptions surveyed [31]. In South Ethiopia, the percentage of encounters in which an antibiotic was prescribed was 58.1% [32]; in Khartoum State (Sudan) 81.3% of prescriptions involved an antibiotic [33], and in India 37% of prescriptions were antibiotics [15].

The leading causes of outpatient visits reported in 2007-2008 in PNG were: malaria, skin diseases, simple cough, pneumonia, diarrhoea, other respiratory diseases, and accidents [34]. Similar findings were evident in this study (Table 2). It is notable that malaria treatment

often included antibiotics, which was not in accordance with the STGs. It is notable that upper respiratory tract infections and simple cough almost always resulted in antibiotics being prescribed. Prescribing antibiotics for conditions for which there is no clinical benefit contributes to the development of antibiotic resistance [35, 36].

No determination can be made from the study data as to the appropriateness of the diagnosis or the selection of antibiotic relating to the health condition of the presenting patient. However, prescriber knowledge/consideration whether the patient is suffering the effects of a bacterial infection, the probable micro-organism involved and the sensitivity of the micro-organism to the antibiotic selected is essential, especially in the absence of pathology facilities in many remote health centres in PNG. Amoxicillin products, chloramphenicol and co-trimoxazole made up approximately 70% of all antibiotics prescribed (Table 3). However, antibiotic selection that was STG non-compliant occurred in one quarter of prescriptions, more frequently written by medical doctors. The reasons for this are unknown but could include a lack of prescriber awareness of the current STGs or because prescribers were aware of unpublished resistance data and/or prescribing was based on personal experience of clinical failures from STG listed antibiotic choices.

The high use of chloramphenicol in PNG is a concern. It is usually reserved for severe typhoid/paratyphoid fever where other antibiotics are unsuitable. It may be used in patients allergic to penicillins or cefalosporins in selected cases such as meningitis, brain abscess, or acute epiglottitis and in rickettsial infections where tetracyclines are unsuitable. Its use in PNG was not for these infections. It can however, cause serious adverse reactions. Its use requires ongoing patient monitoring, which would be difficult to perform in most outpatient settings in PNG.

The level of non-compliant prescribing as assessed against official STGs (Table 4) approximates 40%. However, when the PNG prescriber restriction criteria, correctness of the antibiotic dispensed and the quantity supplied were included in the assessment to consider compliance of antibiotic prescribing against official PNG guidelines, the level increased to approximately 50%. This high level of non-compliant prescribing raises concerns about the use or appropriateness of the STGs, the quality of dispensing and raises questions about the capacity of the supply chain management system that relies heavily on the official STGs and Essential Medicines Lists (EMLs) in PNG. Additionally, the poor ability of some non-medical prescriber categories to correctly calculate children's dosages and antibiotic durations is of concern. The reasons for dispensing inaccuracy may depend on antibiotic stock shortages and competency, as well as satisfying patient requests. It is notable there were occasions when the quantity supplied to the patient considerably exceeded

that prescribed, as well as others that involved undersupply.

Prescriber factors:

In PNG, apart from medical and dental officers, HEOs, NOs, CHWs, and Dental Therapists are permitted to prescribe defined medicines (including antibiotics) for patients. Therefore, as seen in this study, most of the prescribing of antibiotics at LHC was undertaken by CHWs (33.3%) and NOs (48.0%), while MOs performed most of the prescribing at APH (67.6%) and PMGH (92.3%).

The data in Table 5 showed that 39.3% of prescribing by medical prescribers was non-compliant which was mainly associated with antibiotic selection. Overall, non-medical prescribers showed even higher levels of non-compliant prescribing with respect to STGs.

Further studies need to be undertaken to determine the reasons for this, but Cabana et al. outlined a range of factors that could affect prescribers' compliance with STGs [37]. Some possibilities would be lack of: awareness, familiarity, agreement, self-efficacy, support, and a reminder system.

A study by Stark et al. [38] acknowledged the contributions of nurses in the delivery of health care in rural areas of low-income countries with 50-80% of all healthcare professionals being nurses. Nurses performed roles including prescribing medications for which they may or may not have had adequate training, often in the absence of legislation and regulation [39]. This situation is reflected in PNG where much of the antibiotic prescribing at LHC which is a rural health centre was undertaken by nurses (48.0%). LHC, as is similar to all other regional health centres in PNG, is staffed only by non-medical prescribers.

Antibiotic resistance:

Antibiotic resistance can develop more quickly in scenarios where widespread unsupervised access to antibiotics and lack of compliance with STGs is common [5]. Although emphasis internationally is still being placed on the development of updated STGs, which need to reflect current resistance patterns, it is clear that as found in this study it is the lack of adherence to current STGs that is the underlying issue that also needs to be addressed [40]. This is problematic in PNG where the STGs are not updated for many years. Although we strongly support the development and provision of STGs to prescribers they are only a useful tool for prescribers when they contain up to date information. It would seem that although dire warnings of the end of the usefulness of antibiotics have been issued, the methods (prescriber education and information dissemination) still being proposed in 2013 to solve the problem are the same methods that have consistently failed over the past thirty years [41]. There seems little point in educating prescribers to follow STGs that are out of date and lead to treatment failures.

A recent study of antibiotic use in children in outpatient settings in selected locations in PNG

also found high rates of non-compliance with STGs but the rates of return visits for mild pneumonia were similar irrespective of an appropriate or inappropriate antibiotic prescription [9]. This finding needs careful follow-up of patients in several settings to enable patient outcomes to be fully evaluated. These data may also point to the STGs not providing appropriate information.

Limitations:

This study has evaluated the compliance of antibiotic prescribing to official STGs at three sites which represent the main tiers of health care delivery in PNG. We would not consider there would be marked differences at other sites. Data were collected over two week periods but there is no reason to believe these were different from any other period. Drug supplies are delivered every two months and the study was approximately mid-way between deliveries. It is possible that more antibiotic shortages could occur towards the end of a two-month delivery cycle possibly influencing prescribing selections.

CONCLUSIONS:

These results demonstrate unacceptably high levels of antibiotic prescribing and of non-compliant antibiotic prescribing in selected health care provider settings in PNG. Medical prescribers made more non-compliant antibiotic selections while non-medical prescribers made more non-compliant dosage and duration errors. Overall non-compliant antibiotic prescribing was higher in children than adults mainly because of the inability of some non-medical prescribers to calculate dosages with respect to weight. It is important that interventions are performed to reduce antibiotic prescribing and improve the level of dosage and duration prescribing. This can be achieved when regularly updated STGs are available and are monitored. STGs should include antibiotic selections that have documented defined levels of resistance. Resistance patterns in PNG healthcare settings for current antibiotics should be published on an annual basis. The high level of prescribing of oral chloramphenicol in outpatient settings requires review by clinical microbiologists.

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ANTIHYPERGLYCEMIC EFFECTS OF AQUEOUS LEAF EXTRACT OF *SENNA FISTULA* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT:
This study investigated antihyperglycemic effects of chronic administration of aqueous leaf extract of Senna fistula in Streptozotocin-induced diabetic rat. Thirty rats were randomly assigned into six groups (A-F). Animals in group A were the control non-diabetic, in group B were diabetic and received distilled water, in group C were diabetic, treated with 2.5 mg/kg body weight of Glibenclamide, while animals in groups D, E and F were diabetic treated with 28.57, 57.14 and 114.28 mg/kg body weight respectively of aqueous leaf extract of Senna fistula for 28 days. At the end of 28 days blood samples were collected for the assay of Insulin, Superoxide Dismutase, Catalase, and Glutathione Peroxidase in serum and liver Glycogen. The result showed that the blood glucose levels of diabetic rats were significantly reduced in the extract and Glibenclamide treated animals when compared with diabetic rats that received distilled water. Similarly, there was a significant increase in serum Insulin level, Superoxide dismutase, Catalase and Glutathione peroxidase activities and liver glycogen in the extract and Glibenclamide treated diabetic groups when compared with diabetic untreated group. The results indicated that oral administration of aqueous extract of Senna fistula has antihyperglycemic effect by stimulating Insulin secretion and activating antioxidant enzymes.

Keywords: Insulin, Superoxide dismutase, Senna fistula, Glibenclamide, Antioxidant

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disorder caused by several factors. It is characterized by a chronic high level of glucose in the blood due to disorders of protein, carbohydrate and fat, ensuing from defects in insulin secretion, insulin action, or both [1]. The high blood glucose level may occur over a prolonged period and produces the symptoms of polyphagia, polyuria and polydypsia. Untreated, diabetes can lead to many complications [2]. These complications include non-ketotic

hyperosmolar coma and diabetic ketoacidosis which are the acute forms of the complications in Type I DM [3]. Severe long-term complications include damage to the eyes, heart disease, kidney failure, stroke and foot ulcers, which may be due to Type II DM [2].

DM is reaching a pandemic level worldwide and affecting the developing countries of the world much more than the developed countries [4]. In Africa more than 5 million people have been reported to have DM and this figure is expected to rise to 15 million by 2025 [5]. With increased number of people with DM in Africa, the incidence of diabetes complications will also be on the increase equally [6,7]. This may lead to more health care and economic problem, however, with good glycemic control, morbidity and mortality of diabetic patients can be reduced as well as improve their quality of life.

Insulin is the main hormone responsible for controlling the uptake, utilization, and storage of cellular nutrients. Its anabolic action includes stimulation of intracellular utilization and storage of glucose, amino acids and fatty acids while it inhibit breakdown of glycogen, fat and protein mediated through β-adrenoceptor stimulation [8].

Free radicals are highly reactive molecules derived from the metabolism of oxygen; example is reactive oxygen species (ROS) [9]. Some of these free radicals play a positive role in physiological and biochemical processes when present at low/ moderate concentrations. However, over production of these free radicals e.g (ROS) in the body result in oxidative stress [10], causing potential biological damage. The excess ROS can damage cellular proteins, lipids, or DNA, inhibiting their normal function, as a result of this; oxidative stress has been implicated in a numbers of human diseases such as cancer, diabetes, atherosclerosis as well as aging process [10,11]. Inability to remove or destroy excess free radicals (ROS) has been attributed to decrease in endogenous antioxidant enzyme synthesis CAT, SOD, GPx and reduction in non-enzymatic protection (α-tocopherol, ascorbic acid, β carotene and uric acid) [12]. In diabetes, increased oxidative stress is known to be involved in the development and progression of the disease and its complications [13-15]. Therefore, this disease (diabetes) is usually accompanied by increased production of free radicals [14,16,17] or impaired antioxidant defences [18-20]. Studies have shown that a potent scavenger of these free radicals (ROS) may serve as a possible preventive and therapeutic intervention for free radical mediated diseases [21,22].

Traditional medicine has been reported to provide more than 85% of health care in Africa [23]. Similarly in Nigeria, a large segment of the population still rely on medicinal plants and even patronise traditional medicine practitioners for their health care needs [24], and about 46% of people with DM in Nigeria

use herbal remedy in the management of their condition [25].

Several species of plant are on earth [26], of which only small percentages (1-10%) of these are used for food and medicine by human and animals [27]. Medicinal plants have several biologically active compounds such as fat and oil, protein, carbohydrates, enzymes, minerals, vitamins, alkaloids, carotenoids, quinines, terpenoids, flavonoids, sterols, simple phenolic glycosides, tannins, saponins etc. which have medicinal properties.

Senna fistula belongs to the leguminosae family commonly known as Indian laburnum and locally known by the Yorubas as Aidantoroo. The plant has been used significantly in traditional medicine system for the treatment of many diseases, for example the pulps of the ripe fruit have anti-fungal and a mild pleasant laxative effect [28]. The pods are used in the treatment of blood poisoning and malaria. The decoction of the root is applied to treat ulcer and disinfect wound. Other ethno-medical uses of the plant include anti-dysentery and anti-diarrhoea [29]. The plant is also used in the treatment of DM and skin problem [30]. Its hepato-protective and antioxidant effects have also been evaluated [31].

The use of extracts from medicinal plants for the management of DM has received great attention in recent years. The mechanisms underlying their mode of action need experimental verification. At present only few have been verified why thousands are yet to be. This study investigated antihyperglycemic effects of Senna fistula and its mechanisms of action.

MATERIALS AND METHODS:

Plant material and authentication

Senna fistula leaves was purchased from Itoku market in Abeokuta, Ogun state. The identification of the plant leaves was carried out in the Department of Plant Biology of University of Ilorin, Kwara state, Nigeria, with a voucher specimen (UIH 1020), and a specimen was deposited in the Herbarium of the Department. Fresh leaves of S.fistula were air-dried at room temperature for about 2 weeks. The dried leaves were pulverised using electric blender and kept in a plastic container before the commencement of the study.

Animals:

Male and Female rats of Wistar strain, weight between 120 -130 g, were obtained from the animal holding of the Department of Biochemistry, University of Ilorin. The animals were fed on rats pellet (premier feed limited) and water ad libitum. All animals were maintained under standard laboratory conditions of temperature (22± 20C), humidity (45±5%) and natural photoperiod of about 12h light: dark cycle. All rats were handled in accordance with the guide for the care and use of laboratory animals [32].

Glucometer and Assay kit:

One touch ultra-blood glucose meter kit (lifescan, Inc. Milpitas, USA) and Insulin kit (Monobind Inc. Lake forest, USA) were used for this study.

Drugs and chemicals:

Glibenclamide was a product of HOVID Bhd, Ipoh, Malaysia. Streptozotocin and other chemicals were products of Sigma-Aldrich CHEME GMbH, Steinheim, Germany.

Plant extraction:

The aqueous leaf extract of Senna fistula was prepared using the method described by Yakubu et al., with slight modification [33]. About 158.50g of air-dried and powered leaves of S.fitula was exhaustively extracted with 2 litres of water by maceration for 24 hours, after which it was filtered and the filtrate was evaporated to dryness using water bath regulated at 400C. A dark green extract weighing 43.65g (27.54%) was obtained. The extract was stored in the refrigerator before the commencement of the study.

Animal grouping and drug administration:

Rats of both sexes were randomly assigned into 6 groups of 5 rats each:

In Group A were the control (non-diabetic rats); they received 0.5ml of distilled water. In Group B were the streptozotocin- induced diabetic rats; they received 0.5ml of distilled water (untreated rats). Group C were the diabetic rats treated with 2.5mg/kg body weight (bw) of Glibenclamide. Groups D to E were diabetic rats and treated with different doses of the aqueous extract of S. fistula as follows: 28.57mg/kg bw, 57.14mg/kg bw and 114.28mg/kg bw respectively.

The drug and extracts were administered orally, three times daily for a period of 28 days [34].

Induction of diabetes and determination of blood glucose:

The rats were fasted for 18 hours before induction of diabetes. DM was induced by single intraperitoneal injection of freshly prepared streptozotocin (STZ) (50mg/kg bw) in 0.1M citrate buffer (PH 4.5) [35]. The rats were feed (pellet and 5% dextrose saline) one hour after STZ injection to overcome initial hypoglycemic phase [36]. Five days post STZ injection, blood was collected from the tail vein of each rat and DM was confirmed by glucose oxidase method using one touch ultra-blood glucometer. Rats with blood glucose level higher or equal to 12.00mmol/L were used for the study [33].

The blood glucose level was determined using one touch ultra-blood glucometer (lifeScan USA) before the start of the experiment to ascertain their initial blood glucose, after the induction of diabetes and on the last day (day 28) of the experiment.

Insulin was determined using Accu-Bind ELISA kit (Monobind Lake Forest, USA). The assay was carried out using the procedure recommended by the manufacturer.Glycogen content in the liver was determined by the

method of Van [37]. Superoxide dismutase (SOD) activity was determined according to the spectrophotometric method of Mistra and Fridovich [38]. Glutathione peroxidase activity was measured by the procedure of Flohe and Gunzler [39]. Catalase activity was measured using the method of Aebi [40].

Statistical analysis:

All data are expressed as the mean ± Standard error of mean (SEM), the test for significance was done using ANOVA, and Duncan new multiple range test (DMRT). Differences were considered statistically significant at P< 0.05.

RESULTS:

Table 1 depicts the effect of administration of different concentrations of S. fistula extract on blood glucose level of diabetic rats. The diabetic untreated animals had significant high fasting blood glucose (FBG) level. However, the rats treated with different concentrations of Senna fistula extract and Glibenclamide had significant reduction in their fasting blood glucose (FBG) value when compared with diabetic untreated group ($p<0.05$). While there is no significant difference in the fasting blood glucose level of animals treated with different doses of S. fistula and Glibenclamide.

The effects of different concentrations of aqueous extract of Senna fistula on insulin concentration and liver glycogen of diabetic rats are shown in Table 2. There was a significant decrease in insulin level of diabetic untreated animals ($p<0.05$) when compared with the control, extract treated and Glibenclamide treated rats, administration of the extract increased the reduced insulin concentration in diabetic rats which compared favourably with the control and Glibenclamide treated rats. However, there is no significant difference in insulin concentration between the control Glibenclamide and animals treated with different doses of the extract

Similarly, there was a significant decrease in glycogen level of diabetic untreated rats ($p<0.05$) when compared with the control, extract treated and Glibenclamide treated rats, administration of the extract increased the reduced hepatic glycogen concentration in diabetic rats which can be compared with the control and glibenclamide treated rats. There is no significant difference in liver glycogen concentration between glibenclamide and animals treated with different doses of the extract.

Table 3, represents the effect of different concentrations of aqueous extract of S. fistula on catalase, superoxide dismutase and glutathione peroxidase activity of diabetic rats. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) showed a significant decrease ($p<0.05$) in their activities in diabetic untreated rats when compared with the control. Administration of different concentrations of aqueous leaf extract of Senna fistula reversed this trend significantly

(p<0.05). However activity of catalase was more in glibenclamide treated group, while, there were no significant difference (p<0.05) in SOD and GPX activities between the glibenclamide and all the extract treated rats.

Table 1: Effect of administration of aqueous leaf extract of S. fistula on fasting blood glucose level of STZ-induced diabetic rats

	Fasting blood glucose (mmol/L)	
Groups/Days	Initial (Post induction)	Final (After 28 days)
Control	4.71±0.45a	4.26±0.38a
Diabetic untreated	23.82±2.56b	30.28±3.52b
Diabetes + Glibenclamide	20.66±2.98b	3.66±0.39a
Diabetes + 28.57 mg/kg bw	33.80±0.00c	6.24±1.14a
Diabetes + 57.14 mg/kg bw	22.22±2.78b	4.76±0.19a
Diabetes + 114.57 mg/kg bw	23.10±2.98b	6.02±0.83a

Values are expressed as Mean ±SEM, n= 5. Mean with different letters are significantly different (p<0.05)

Table 2: Effect of administration of aqueous leaf extract of Senna fistula on insulin and liver glycogen level of streptozotocin-induced diabetic rats

Group/parameters	Insulin (µU/ml)	Glycogen (mg/100g tissue)
Control	10.50±0.63b	6.59±0.25d
Diabetic untreated	5.50±0.87a	1.36±0.14a
Diabetes + glibenclamide	8.75±0.75b	2.39±0.18b
Diabetes + 28.57mg/kg bw	9.50±2.24b	3.23±0.35bc
Diabetes + 57.14mg/kg bw	10.20±0.60b	3.81±0.38c
Diabetes + 114.57mg/kg bw	10.13±1.96b	3.76±0.44c

Values are expressed as Mean ±SEM, n= 5. Mean with different letters are significantly different (p<0.05)

Table 3: Effect of S. fistula leaf extract on antioxidant enzymes of STZ-induced diabetic rats

Group/parameters	Catalase (U/mg protein)	Superoxide dismutase (U/mg protein)	Glutathione peroxidase (U/mg protein)
Control	3.87±0.52c	15.57±3.11c	0.14±0.21c
Diabetic untreated	0.76±0.10a	1.69±0.22a	0.04±0.12a
Diabetes + glibenclamide	7.47±0.83d	8.35±0.17b	0.11±0.14bc
Diabetes + 28.57mg/kg bw	1.90±0.31b	6.16±1.60b	0.08±0.31bc
Diabetes + 57.14mg/kg bw	1.95±0.34b	8.83±2.08b	0.05±0.11b
Diabetes + 114.57mg/kg bw	2.95±0.22bc	5.50±2.42b	0.12±0.25c

Values are expressed as Mean ±SEM, n= 5. Mean with different letters are significantly different (p<0.05)

DISCUSSION:

Plants have been a good source of drugs and most available drugs have been obtained directly or indirectly from plant. There are different types of blood glucose lowering drugs, exerting antidiabetic effects through different mechanisms, for example alpha glucosidase act by delaying the intestinal absorption of glucose, also sulphonylurea acts by stimulating insulin secretion while thiazolidinediones act by increasing peripheral uptake of glucose [41]. Anti-hyperglycemic effects of plants are mainly due to their ability to restore and enhance the functions of pancreatic tissue either by causing an increase in insulin output or inhibit intestinal absorption of glucose or reduce hepatic gluconeogenesis [42]. Antidiabetic potential of medicinal plants have been grouped into two; the primary antidiabetic potential which refers to biological activity which have hypoglycemic effects through the actions of insulin producing and insulin responsive cells required for glucose, protein and lipid homeostasis, and the secondary antidiabetic potential involving protection against long term diabetic complications through their antiglycation and antioxidant properties [43]. The significant reduction in the blood glucose level of diabetic rats treated with different doses of S. fistula extract suggests anti-hyperglycemic effect of the plant.

The anti-hyperglycaemic effect of the S. fistula extract may be partly linked to its constituents that have been reported to contain glycosides, flavonoids, terpenoids, Ca, K, Zn, Mn, Mg and Vitamin C [44]. Studies have reported that medicinal plants with anti-hyperglycemic property usually contain flavonoids, tannins, terpenoids and Alkaloids [45,46]

Beta-cells of the pancreas are selectively destroyed by a cytotoxic substance like streptozotocin (STZ) which cause a significant reduction in the synthesis of endogeneous insulin [47]. Glibenclamide on the other hand stimulate the release of insulin from the remaining pancreatic β cells [8]. In STZ-induced diabetic animals, serum insulin level was found to decrease whereas upon administration of the extract, there was a significant increase in serum insulin level in diabetic rats which is comparable to Glibenclamide treated rats. This finding agrees with the study of Einstein et al., [48], where it was documented that methanolic extracts of Cassia fistula bark and leaf caused significant increase in plasma insulin in diabetic rats. Improvement in insulin secretion in extract treated groups signify a pancreatic mode of action of S. fistula, which may be related to the presence of some minerals and secondary metabolites like Zinc, K, Mn, Mg, Terpenoids, Flavonoids, Glycosides and others. This

observation is supported by findings that natural products classified into Flavonoids, Terpenoids, Phenolics, Alkaloids etc exhibit antidiabetic potentials through insulinomimetic activity [42]. Similarly, minerals like K, Ca Zn, Mn Fe etc have been reported to stimulate insulin secretion from the beta cells of the pancreas [49,50]. Therefore it can be deduced that the Senna fistula leaf extract might have acted as an antihyperglycaemic agent by stimulating the release of insulin from the remnant pancreatic β cell [51].

Liver glycogen level is often used to assess antihyperglycaemic activity of any dug. In this study the significant decrease in liver glycogen of the diabetic untreated rats agrees with previous work documenting a decrease in liver glycogen in diabetic rats [52], which may be due to increase in glucose output due to insulin deficiency. The decrease in liver glycogen in diabetic rats may also be linked to decrease in insulin level because glycogen level in the liver is a direct reflection of insulin activity. Insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Aqueous extract of S. fistula leaves increased the lowered liver glycogen in diabetic rats. The extract might have acted by reactivating glycogen synthase system as a result of increased insulin secretion [53] or due to insulinomimetic effect of the extract leading to increased peripheral glucose uptake [54, 55].

The significant decrease in the level of the activities of CAT, SOD and GPx in STZ-induced diabetic rats may be associated with increased oxidative stress and / or decrease antioxidant defence potentials, because diabetes is usually accompanied by increase production of free radicals [14,16,17], or impaired antioxidant defences [18-20]. The improvement in the activity of CAT, SOD, GPx following treatment with aqueous extract of S. fistula and Glibenclamide agree with studies documenting antioxidant effects of this plant in diabetic rats [56], which indicate the possible mechanisms of the beneficial effects of S. fistula in the treatment of DM. This antioxidant activity may be due to the reduction in the imbalances between ROS production and scavenging enzymes actively in diabetic rats. The extract of S. fistula leaves may act either by directly scavenging reactive oxygen metabolites due to the presence of various antioxidant compounds [57] or by increasing the synthesis of antioxidant.

This study concludes that the significant reduction of the FBG in all the extract treated groups to the value of the control and the reference treated groups signifies anti hyperglycemic property of Senna fistula.

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APHRODISIAC EFFECTS OF AQUEOUS EXTRACTS OF *Pausinystalia yohimbe, Cassia sieberiana* and *Cissus populnea* ROOTS IN PAROXETINE-INDUCED SEXUAL DYSFUNCTION MALE RATS: A COMPARATIVE STUDY

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ABSTRACT

There is a folkloric claim that *Pausinystalia yohimbe, Cassia sieberiana and Cissus populnea* roots can be used to enhance sexual behaviour in male rats. However, there is still dearth of scientific evidence that substantiated the acclaimed efficacy of separate and combined use of the plant as sex enhancer. Therefore, the aims of this study were to compare the separate and combined effects of aqueous extracts of *Pausinystalia yohimbe, Cassia sieberiena and Cissus populnea* roots in paroxetine-induced sexually impaired male rats. Thirty five male rats were assigned into seven groups (A-G) such that rats in group A received orally 1.0 ml of distilled water for 7 days, while those in groups B - G which were induced into sexual dysfunction (administration of 10 mg/kg of paroxetine) also received equal volume of distilled water, 7.14 mg/kg body weight of PowmaxM (a reference drug), 50 mg/kg body weight of *P. yohimbe*, 50 mg/kg body weight of *C. sieberiana*, 50 mg/kg body weight of *C. populnea* and 50 mg/kg body weight of 1:1:1 mixture of the three extracts, once daily for seven days respectively. The sexual behavior indices of the male rats and the levels of their reproductive hormones were evaluated by standard procedures. The paroxetine-treatment related reductions ($P<0.05$) in the sexual behaviour indices of Mount Frequency, Intromission Frequency and Ejaculatory Frequency, levels of serum reproductive hormones of testosterone, luteinizing hormone and follicle stimulating hormone were progressively attenuated by the separate administration of the plant extracts. Furthermore, the increases in the Mount Latency, Intromission Latency, Ejaculatory Latency and Post-ejaculatory Interval were also gradually reduced, following the administration of the plant extracts. The male rat sexual behaviour indices and the levels of the male reproductive hormones following the administration of the 1:1:1 mixture of the extracts were not significantly different ($P>0.05$) from the effects of the separate extracts. All these changes compared favourably ($P>0.05$) well with those of the sexual dysfunction rats that received PowmaxM (Group G). The results obtained in the present study indicate that the extracts of these plants may have the potential for the management of sexual dysfunction in male rats. The combined use of the plants was not significantly better than the individual use of the plants thereby, each and any of the three plants readily available might be used for this purpose.

Keywords: Aphrodisiac; *Pausinystalia yohimbe; Cassia sieberiana; Cissus populnea*; Paroxetine; PowmaxM; male sexual dysfunction

INTRODUCTION:

A normal male sexual response cycle is functionally divided into five interrelated events, which include libido, erection, orgasm, ejaculation and detumescence. These events must occur in a defined sequence for a normal sexual function [1]. The disturbance during any phase of the normal sexual response cycle that prevents the male from experiencing satisfaction from sexual activity is termed male sexual dysfunction (MSD). Sexual dysfunction can occur as a result of physical causes (neurogenic disorders - spinal cord and brain injuries, nerve disorders such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, and stroke; hormonal disorders - pituitary gland tumor; low level of testosterone; lifestyle – alcohol and drugs, obesity, cigarette smoking [2,3]; and non-physical causes: mental disorders (clinical depression, schizophrenia, substance abuse, panic disorder, generalized anxiety disorder, personality disorders or traits, psychological problems, anxiety, work-related stress negative feelings [3]. Although, MSD have been managed with different strategies (including surgical and non-surgical approaches), the adverse effect/limitations of treatments, together with the high cost of treatment and drugs, have led to several research works on the search for natural treatment options that will not only increase sexual potency and sexual pleasure, but also are affordable, readily available, fast acting and with little or no side effects. In Nigeria, the use of herbal remedies in enhancing normal functioning of the male reproductive organs, and strengthening erection and sex-drive have been reported [4,5,6,7,8]. *Pausinystalia yohimbe, Cassia sieberiena* and *Cissus populnea* are another set of medicinal plants widely acclaimed to be of immense importance in the management of sexual dysfunction in Nigeria.

Cissus populnea Guill & Perr (Vitaceae), also known locally as *Ogbolo* or *Ajara* (Yoruba), *Daafaaraa* or *Latutuwa* (Hausa) and *Okoho* (Igbo) is a strong woody plant 8-10m long, by 7.5cm by diameter, which is dispersed across West Tropical Africa [9]. Phytochemical screening of the stem and root extracts revealed the presence of alkaloids, cardiac glycosides, anthraquinones, phytosteroids, tannins, flavonoids, saponins, and cyanogenic glycosides [10]. The leaves are reported to be used as thickening agent in soup for postnatal stoppage of bleeding [10], while the roots had also been reported to be used by the Yorubas to cure sore breasts of women at childbirth, and as a male coital adjunct [9]. Its stem extracts have been credited with antibacterial and antitrypanosomal properties [11-12] and as a component of a Nigerian anti-sickling herbal formula [13].

Cassia sieberiana (Caesalpiniaceae), also known locally as *Aridan toro* (Yoruba), *Apagban* (Bini), *Gama fada* (Hausa) is a

savannah tree that usually grows from 2 - 12 metres tall and mostly found in the dry areas of the forest and thickets. The phytochemical screening of the plants root and stem bark extract revealed the presence of alkaloids, anthraquinones, flavonoids, triterpenoids, tannins, cardiac glycosides, saponins, reducing sugars and other carbohydrates [14]. *Cassia sieberiana* has many medicinal usages. The roots are used as a diuretic and vermifuge that can be used to treat diseases such as elephantiasis, leprosy, diarrhea, heamorrhoids, dysentery and venereal diseases [15], while the leaves have been reported to help with the symptoms of arthritis and rheumatism. The extracts are used to treat fever, malaria, diuretics, diarrhoea, leprosy, The aqueous extracts of the roots, stem, bark and the fruit pulp have been used traditionally in North-eastern Nigeria for the management of malaria [14], ulcer [16], inflammatory conditions, tiredness and joint pains [17], bilhazia, stomach pains and as a dewormer [18]. *Pausinystalia yohimbe* (K. Schumann) Pierre ex Beille (Rubiaceae), formerly known as *Corynanthe yohimbe* and sometimes spelled johimbe, is also known locally as *takitaki* (Yoruba) and the tree grows about 30m tall, with a straight bole that is rarely larger that 50-60 cm in diameter. It is a psychoactive plant which contains the tryptamine alkaloid- yohimbine that has been used primarily in the treatment of sexual dysfunction, weight (body fat) loss, and xerostomia (dry mouth) [19]. It has also been used in studies investigating autonomic failure and orthostatic hypotension. It is widely distributed over-the-counter as an herbal aphrodisiac. It has been purported to be helpful for men with erectile dysfunction (ED) and for sexual side effects caused by some antidepressants (SSRIs) [19, 20]. In addition to yohimbine, the tree also contains 55 other alkaloids. Yohimbine accounts for 1-20% of its total alkaloid content. Among the others is corynanthine, an alpha-1 adrenergic receptor blocker [21]. Hence, the use of yohimbe extract in sufficient dosages was reported to provide concomitant alpha-1 and alpha-2 adrenoceptors blockade and thus may enhance erections than yohimbine alone [22].

We were informed from the ethnobotanical survey that using only one or two of these plants extracts separately to determine its aphrodisiac activities may not yield effective result. Therefore, the aims of this study were to compare the separate and combined effects of aqueous extracts of *Pausinystalia yohimbe, Cassia sieberiena* and *Cissus populnea* roots in paroxetine-induced sexually impaired male rats.

MATERIALS AND METHODS:

Dried roots of *Cissus populnea, Cassia sieberiana* and *Pausynistalia yohimbe* free from fungal infection or other contaminants, obtained from a farmland through the help of a herb seller at a market (Oja tuntun) in Ilorin, Nigeria, were authenticated at the University of Ilorin

Herbarium, Ilorin, Nigeria, where voucher samples (UIH 1019, UIH 619 and UIH 819 respectively) were deposited. Thirty five, healthy, Wistar, male rats and same number of female rats were housed in clean aluminum cages contained in well ventilated housing conditions (temperature: 22 ± 3oC; photoperiod: 12 hours light/dark phase; humidity: 45-50%). The rats were allowed free access to rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water. The study was conducted following an ethical clearance from the Ethical Committee on the care and use of laboratory animals of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Paroxetine hydrochloride was a product of S.C. Europharm, Brasov, Romania, while PowmaxM was from Beijing Kowloon Pharmaceuticals Co., Ltd, Beijing, China. Progesterone was a product of Ningbo Tisun Medic Biochemic Co., LTD., Ningbo, Peoples Republic of China, while Estradiol benzoate was from Sigma Chemical, St. Louis, USA. Assay kits for testosterone, follicle stimulating hormone and luteinizing hormones were products of Inteco Diagnostics UK LTD., London, United Kingdom. All other reagents used were of analytical grade and were prepared in distilled water and stored in neat and airtight reagent bottles.

Preparation of aqueous extracts of *Cissus populnea, Cassia sieberiana* and *Pausinystalia yohimbe* roots: A known weight (300 g) of the roots of *Cissus populnea, Cassia sieberiana* and *Pausinystalia yohimbe* were separately washed, sliced, oven-dried and pulverized in a blender (Mikachi Blender, Model MK-1830, China) after which the resulting powder (100 g) was extracted in 200 ml of distilled water for 48 hours at room temperature with constant shaking. A dark brown coloured extract was obtained for *Cissus populnea, Cassia sieberiana* and *Pausinystalia yohimbe*. The extracts were filtered separately with Whatman No. 1 filter paper (Maidstone, England) and the resulting filtrate was concentrated on steam bath to give a yield of 5.25 g, 5.75 g and 6.04 g for *Cissus populnea, Cassia sieberiana* and *Pausinystalia yohimbe* respectively. Calculated amounts for each extract were reconstituted in distilled water to give the required doses of 50 mg/kg body weight and also a composite mixture of the three extract in ratio 1:1:1 to also give a required dose of 50 mg/kg body weight.

Induction of sexual dysfunction and assessment of mating behaviour indices in male rats: Thirty male rats were each induced with sexual dysfunction by oral administration of 10 mg/kg of paroxetine hydrochloride suspension (prepared daily in Tween-80 [BDH Chemicals, Ltd., Poole, England], suspended in 0.9% saline solution) using a metal oropharyngeal cannula [23, 24]. Healthy female rats were made receptive by sequential subcutaneous administration of oestradiol benzoate (10 µg/100 g body weight) and progesterone (0.5 mg/100 g body weight), 48 and 4 hours respectively prior to pairing [25]. Oestrus phase in female rats was confirmed by

vaginal smears examinations according to the Organisation for Economic Co-operation and Development (OECD) - 106 guidelines [26]. The oestrous female rats were then introduced into the male rats in their respective cages and observed for 30 minutes for mating behavior of Mount Frequency (MF: number of mounts without intromission from the time of introduction of the female until ejaculation), Intromission Frequency (IF: number of intromissions from the time of introduction of the female until ejaculation), Ejaculation Frequency (EF: number of ejaculations made during the observatory period), Mount Latency (ML: time interval between the introduction of the female and the first mount by the male), Intromission Latency (IL: time interval between the introduction of the female to the first intromission by the male, usually characterized by pelvic thrusting and springing dismounts), Ejaculation Latency (EL: time interval between the first intromission and ejaculation, usually characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity or reduced activity), and Post-Ejaculation Interval (PEI: time interval between ejaculation and erection of the male copulatory organ for the next phase) [4]. For the interpretation of the data, male rats that showed minimum of 25% reduction in MF, IF, and EF as well as a minimum increase of 25% in ML, IL, and PEI were considered as sexually impaired and were used for the subsequent study [7, 24]. Thus, sexual dysfunction being referred to in the present study included the testosterone-dependent behavior of mating, copulation, and ejaculation.

Animal Grouping and Extract Administration:

A total of 35 male rats that were acclimatized for 2 weeks were assigned into seven Groups (A-G) in a complete randomized design, with each group comprising five rats as follows: In Group A (control group) were normal rats that received distilled water. Group B were Sexual dysfunction rats administered distilled water. Group C were Sexual dysfunction rats administered 7.14 mg/kg body weight of PowmaxM (this is the reference male sexual stimulant and energy enhancing polyherbal drug made up of *Panax ginseng, Camelia sinensis, Cnidium monnieri, Epimedium brevicornum, Songaria cynomorium, Gingko biloba, Dahurian angelica, Salvia miltiorrhiza* root, L-arginine hydrochloride, and gamma aminobutyric acid) [27]. In Groups D, E, F and G were Sexual dysfunction rats administered 50 mg/kg body weight of *Pausinystalia yohimbe, Cassia sieberiana, Cissus populnea* and a composite mixture of the three extracts in ratio 1:1:1 respectively.

The rats in the various groups were orally administered 1.0 ml each of distilled water, PowmaxM and the extracts, once daily (08:00 - 08:45 am) for 7 days, with the aid of a metal oropharyngeal cannula. The rats were maintained on unrestricted access to rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water. The male sexual behavior parameters

were monitored and results were recorded 30 minutes after dosing on days 1, 3, and 7 (between 17:00 and 21:00 h) under dim light condition at room temperature (26–28°C).

The blood from the rats was collected into clean, dry centrifuge tubes. The samples were left undisturbed for 15 min at room temperature for coagulation to take place. Clear serum was then collected using Pasteur pipette after centrifuging at 33.5 × g for 15 min using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, UK). The sera were kept frozen for 12 h before being used for the various hormonal assays.

The concentrations of the hormones, testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in serum were quantitatively determined using the direct human serum enzyme immunoassay kits as outlined in the manufacturer's manual [28]

Data were expressed as the mean ± SEM of five determinations. Means were analyzed using one-way analysis of variance followed by Duncan Multiple Range Test. Statistical Package for Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Differences were considered statistically significant at $P < 0.05$.

RESULTS:

Administration of paroxetine significantly decreased ($P<0.05$) the MF, IF and EF while the ML, IL, EL, and PEI were significantly increased as compared to the control (Table 1).

The changes in all the sexual behaviour parameters investigated were more than 25 % (Table 1). Administration of the aqueous extracts of *P. yohimbe, C. sieberenia* and *C. populnea* to sexually impaired male rats significantly increased the MF and IF of the rats on Days 3 and 7 (Table 2). The improvement compared significantly with the reference drug (PowmaxM) but the sexual function of the rats was not restored back to normal when compared with the non-sexually impaired rats administered distilled water (Table 2).

Administration of the mixture of the extracts and reference drug significantly ($P < 0.05$) increased the EF of the sexually impaired male rats on Day 1 while all the extracts as well as the mixture significantly increased the EF on Days 3 and 7 (Table 3). The ML of sexually impaired male rats administered aqueous extract of *P. yohimbe, C. sieberenia* and *C. populnea* as well as the mixture of the extracts were significantly increased (Table 3). The increase compared significantly with the reference drug (PowmaxM) but did not compare significantly with the control rats that were administered distilled water only (Table 3). Administration of the extracts and their mixture significantly reduced the IF and EF of the sexually impaired male rats in days related manner which compared significantly with the reference drug treated rats (Table 4). This trend of reduction was extended to the PEI but did not compare significantly with non-sexually impaired rats administered distilled water group

(Table 5). The serum concentrations of testosterone and luteinizing hormone significantly decreased in sexually impaired male rats while the concentration of follicle stimulating hormone significantly increased when compared with non-sexually impaired rats administered distilled water group only (Table 6). Administration of *P. yohimbe, C. sieberiana* and *C. populnea* separately and combined at 50mg/kg body weight significantly increased ($p<0.05$) the concentrations of testosterone and luteinizing hormone in a manner similar to the reference drug while the serum concentration of follicle-stimulating hormone significantly decreased (Table 6).

Table 1: Effect of paroxetine administration on sexual behaviours of male rats

Parameters	Control	Paroxetine-treated rats	Percentage change (%)
Mount frequency (MF) (numbers)	13.36±0.86[a]	5.08±0.21[b]	61.97#
Intromission frequency (IF) (numbers)	11.46±0.12[a]	4.88±0.23[b]	57.41#
Ejaculatory frequency (EF) (numbers)	1.83±0.18[a]	1.06±0.13[b]	42.08#
Mount latency (ML) (seconds)	101.61±3.35[a]	167.26±5.33[b]	64.61+
Intromission latency (IL) (seconds)	132.33±4.11[a]	196.41±3.54[b]	67.37+
Ejaculatory latency (EL) (seconds)	147.18±6.53[a]	226.68±5.49[b]	64.93+
Post-ejaculatory interval (PEI) (seconds)	186.54±5.82[a]	241.93±7.34[b]	29.69+

Data are mean of five determinants ± SEM.
Values carrying superscripts different from the control for each parameter are significantly different ($P<0.05$)
means percentage reduction in parameter. + means percentage increase in parameters

Table 2: Effect of aqueous extract of *P. yohimbe, C. sieberiana* and *C. populnea* roots on mount and intromission frequencies of paroxetine-induced sexual dysfunction male rats

Treatments	Mount frequency (numbers)			Intromission frequency (numbers)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Distilled water (control)	12.98±0.76[a]	13.41±0.61[a]	13.36±0.59[a]	11.33±1.03[a]	11.81±0.74[a]	10.83±0.65[a]
Paroxetine + Distilled water	5.19±0.19[b]	5.33±0.22[b]	5.84±0.23[b]	4.93±0.31[b]	5.02±0.18[b]	5.47±0.22[b]
Paroxetine + *P. yohimbe*	5.39±0.26[b]	7.68±0.91[c]	9.76±0.70[c]	5.02±0.51[b]	8.80±0.33[c]	8.85±0.31[c]
Paroxetine + *C. sieberiana*	5.54±0.34[b]	7.92±0.38[c]	9.28±0.54[c]	5.11±0.48[b]	8.85±0.35[c]	9.01±0.37[c]
Paroxetine + *C. populnea*	5.26±0.41[b]	8.21±0.43[c]	10.11±0.81[c]	5.04±0.26[b]	8.98±0.47[c]	8.97±0.33[c]
Paroxetine + extract ABC	5.29±0.28[b]	9.11±0.36[d]	10.18±0.76[c]	5.06±0.50[b]	8.57±0.58[c]	9.26±0.46c
Paroxetine + PowmaxM	5.32±0.30[b]	8.57±0.50[d]	9.63±0.33[c]	5.05±0.49[b]	9.11±0.81[c]	8.89±0.35[c]

Data are mean of five determinants ± SEM. Values carrying superscripts different from the control down the group for each day and parameter are significantly different ($P<0.05$).

Table 3: Effect of aqueous extract of *P. yohimbe, C. sieberiana* and *C. populnea* roots on ejaculation frequency and mount latency of sexual dysfunction male rats

Treatments	Ejaculation frequency (numbers)			Mount Latency (seconds)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Distilled water (control)	2.00±0.00[a]	2.25±0.25[a]	2.25±0.29[a]	114.18±3.12[a]	120.88±3.55[a]	116.04±4.36[a]
Paroxetine + Distilled water	1.25±0.25[b]	1.25±0.25[b]	1.25±0.29[b]	160.64±4.64[b]	154.23±2.11[b]	152.16±5.84[b]
Paroxetine + *P. yohimbe*	1.25±0.25[b]	1.50±0.29[c]	1.75±0.29[c]	156.54±5.33[b]	146.52±4.81[c]	131.05±4.53[c]
Paroxetine + *C. sieberiana*	1.25±0.25[b]	1.50±0.29[c]	1.75±0.25[c]	153.61±4.98[b]	142.53±4.30[c]	128.10±3.61[c]
Paroxetine + *C. populnea*	1.25±0.25[b]	1.75±0.25[d]	1.75±0.25[c]	158.32±5.21[b]	143.38±6.49[c]	135.20±4.85[c]
Paroxetine + extract ABC	1.50±0.29[c]	1.50±0.29[c]	1.75±0.29[c]	151.19±5.91[b]	143.90±5.77[c]	130.13±3.91[c]
Paroxetine + PowmaxM	1.50±0.29[c]	1.50±0.29[c]	1.75±0.29c	153.84±4.63[b]	144.37±6.85[c]	125.80±1.68[d]

Data are mean of five determinants ± SEM. Values carrying superscripts different from the control down the group for each day and parameter are significantly different (P<0.05).

Table 4: Effect of aqueous extract of *P. yohimbe, C. sieberiana* and *C. populnea* roots on intromission and ejaculatory latencies of paroxetine-induced sexual dysfunction male rats

Treatments	Intromission Latency (seconds)			Ejaculatory Latency (seconds)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Distilled water (control)	129.84±3.66[a]	132.34±3.22[a]	127.80±4.43[a]	153.31±6.24[a]	148.27±4.41[a]	143.53 ±5.39[a]
Paroxetine + Distilled water	173.92±4.19[b]	168.90±7.10[b]	165.32±5.21[b]	195.39±4.89[b]	186.47±6.88[b]	179.92±6.36[b]
Paroxetine + *P. yohimbe*	172.36±3.81[bc]	151.05±5.67[c]	142.50±5.88[c]	188.99±5.31[c]	168.37±5.33[c]	153.70±4.48[c]
Paroxetine + *C. sieberiana*	168.45±5.12[bc]	154.56±6.11[c]	139.15±4.89[c]	188.34±4.63[c]	164.54±4.61[c]	148.95±5.97[c]
Paroxetine + *C. populnea*	165.75±4.93[bc]	153.26±5.34[c]	141.23±5.31[c]	189.63±6.14[c]	166.44±8.11[c]	151.46±6.27[c]
Paroxetine + extract ABC	162.19±4.61[c]	151.09±6.38[c]	144.33±4.64[c]	182.28±4.15[d]	168.37±7.16[c]	154.12±6.83[c]
Paroxetine + PowmaxM	164.36±4.81[c]	155.13±5.89[c]	140.41±4.84[c]	191.65±5.43[c]	165.80±6.18[c]	153.82±5.81[c]

Data are mean of five determinants ± SEM. Values carrying superscripts different from the control down the group for each day and parameter are significantly different (P<0.05).

Table 5: Effect of aqueous extract of *P. yohimbe, C. sieberiana* and *C. populnea* roots on Post-ejaculatory interval of paroxetine-induced sexual dysfunction male rats

Treatments	Post-ejaculatory Latency (seconds)		
	Day 1	Day 3	Day 7
Distilled water (control)	193.61±6.27[a]	185.34±5.83[a]	183.06±3.61[a]
Paroxetine + Distilled water	238.68±6.49[b]	232.24±7.13[b]	239.40±9.35[b]
Paroxetine + *P. yohimbe*	236.07±5.87[b]	224.67±6.06[c]	194.54±8.89[c]
Paroxetine + *C. sieberiana*	232.65±6.22[c]	224.54±7.46[c]	193.51±7.59[c]
Paroxetine + *C. populnea*	235.53±5.45[b]	221.14±6.93[c]	190.13±8.36[c]
Paroxetine + extract ABC	230.46±5.81[c]	220.33±7.04[c]	195.88±7.68[c]
Paroxetine + PowmaxM	232.91±6.11[c]	221.30±8.01[c]	190.11±7.26[ac]

Data are mean of five determinants ± SEM. Values carrying superscripts different from the control down the group for each day and parameter are significantly different (P<0.05).

Table 6: Effect of aqueous extract of *P. yohimbe, C. sieberiana* and *C. populnea* root on serum concentrations of reproductive hormones in sexual dysfunction male rats

Treatment	Testosterone (nmol/l)	Follicle-stimulating hormone (mlu/ml)	Luteinizing hormone (mlu/ml)
Distilled water (control)	6.60±0.42[a]	5.60±0.22[a]	3.50±0.15[a]
Paroxetine+Distilled water	2.80±0.11[b]	6.78±0.26[b]	2.65±0.11[b]
Paroxetine + *P. yohimbe*	4.80±0.27[c]	4.90±0.18[c]	4.50±0.16[c]
Paroxetine + *C. sieberiana*	4.60±0.14[c]	4.75±0.21[c]	4.60±0.23[c]
Paroxetine + *C. populnea*	4.50±0.15[c]	4.60±0.14[c]	5.50±0.35[d]
Paroxetine + extract ABC	4.50±0.05[c]	4.70±0.10[c]	3.80±0.24[e]
Paroxetine + PowmaxM	4.90±0.17[c]	4.50±0.17[c]	4.20±0.16[c]

Data are mean of five determinants ± SEM. Values carrying superscripts different from the control down the group for each day and parameter are significantly different (P<0.05).

DISCUSSION:

The sexual behaviour parameters of MF, IF and EF were decreased while the ML, IL, EL and PEI were prolonged following the administration of paroxetine to sexually active male rats in our present study. These alterations could be due to the capability of selective serotonin reuptake inhibitors (SSRIs) such as paroxetine to decrease libido and cause other sexual side effects by increasing synaptic concentrations of serotonin and stimulating 5HT2, and possibly 5HT3, receptors [29]. The increase in MF and IF of sexually impaired rats following the administration of the

aqueous root extracts of *Pausinystalia yohimbe, Cassia sieberiana* and *Cissus populnea* shows enhanced sexual desire [30, 31]. This could be due to the action of components in the extract to reduce the synaptic concentration of serotonin as well as increase the amount of dopamine release [32].

The increase recorded in ML, IL and EL, indicators of sexual arousability and potency, in sexually impaired rats could have resulted from reduced erectile response through inhibition of the synthesis of nitric oxide in the penis leading to reduced carvenous stimulation in paroxetine-treated rats [33, 34, 35]. The action of components in the extracts and their mixture to reverse these increases in a manner similar to the reference drug (PowmaxM) indicates their potential to stimulate sexual arousability, performance, motivation, and vigour in the rats [36]. The PEI, an index of potency, libido, and rate of recovery from exhaustion after first series of mating [37], was elevated in paroxetine-induced sexual dysfunction rats which indicate loss of libido and potency [4]. Following the administration of the extracts as well as their mixture to the sexually impaired rats, the PEI was attenuated which denotes enhanced libido and potency. These recoveries from paroxetine-induced sexual dysfunction in sexually impaired rats were similar to the recovery pattern in the reference drug treated groups. The positive effects on the indices of male sexual behaviour may have been due to the actions of constituents of the extracts on testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) assessed in the present study. Therefore, there is a need to further evaluate the effects of administration of the extracts on serum concentrations of these hormones [38] as they are hormonal markers of androgenicity [39].

The results obtained in the present study indicate that the extracts of these plants may have potential for the management of sexual dysfunction in male rats. The combined use of the plants was not significantly better than the individual use of the plants thereby, each and any of the three plants readily available might be used for this purpose.

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LICORICE AMELIORATES IMBALANCE BETWEEN REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYMES IN THE BRAIN OF SLEEP DEPRIVED RATS.

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*Running title: Effect of licorice on brain oxidative stress markers in sleep deprived rat*s.
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ABSTRACT

Sleep deprivation can be described as inadequate quantity or quality of sleep characterized by insufficient sleep duration, delayed sleep onset, and occurrence of napping episodes during the day. Sleep deprivation in animals and obstructive sleep apnea syndrome in human was reported to be associated with increased oxidative stress. *Glycyrrizha glabra* (licorice) is a medicinal plant known to be a highly efficacious medicinal herb with several pharmacological effects. Hence, the aim of this study was to demonstrate whether or not licorice root extract will regulate the imbalance between the reactive oxygen species and production of antioxidant enzymes in the brain of sleep deprived rats. Twenty - five 6-week-old male Wistar rats were randomly divided into five groups to undergo sleep deprivation and recovery for 5 days each. Group I (Control): Group II: sleep deprivation (SD); Group III: sleep deprivation and recovery (SD+SR) all received distill water (10ml/kg) orally; Group IV: sleep deprivation and licorice (SD+Lic), Group V: sleep deprivation, recovery with licorice (SD+SR+Lic) both received licorice (150mg/kg) orally once daily. MDA concentration among rats in Groups II (51%), III (46.7%) and IV (31.3%) were significantly higher when compared with control. Rats in Group III (20.5%), Group IV (24.6%) and Group V (30.8%) showed increased significant change in GSH concentration when compared with Group II. The concentration of CAT among rats in Group II was significantly lower than those rats in Group III (43.8%), Group IV (53.8%) and Group V (72.9%). These results clearly show that sleep deprivation significantly affects the oxidative status of rats. In conclusion, licorice root extract has ameliorative effect on the imbalance between the reactive oxygen species and production of antioxidant enzymes in the brain of sleep deprived rats.

Keywords: Sleep; Sleep deprivation; Licorice; Oxidative stress; Rats

INTRODUCTION:

Sleep is important for proper brain function, no less than air, water, and food [1]. However, sleep from this perspective could be defined on the basis of both the behavior of the person while asleep and related physiological changes that occur to the waking brain's electrical rhythms during sleep [2]. Despite the need by individuals to stay awake for long hours due to work or study, sleep remains an integral part of human health and is crucial for learning, performance, physical and mental health [2].

Sleep deprivation (SD) can be described as inadequate quantity or quality of sleep characterized by insufficient sleep duration, delayed sleep onset, and occurrence of napping episodes during the day [1]. It is an increasingly common occurrence in modern life which predisposes humans to many diseases as a result of immune system deficiency, endocrine deregulation and oxidative stress (OS) [3]. While sleep has important functions for every organ in the body sleep deprivation leads to disorders that cause irreparable damage [4]. Furthermore, sleep loss was also reported to be associated with adverse health effects such as obesity, type 2 diabetes, hypertension, and cardiovascular disease [5]. Sleep deprivation in humans and rats was also reported to promote increase in food intake [6]. In contrast, previous studies reported that sleep deprived animals showed intense catabolism [7] and energy expenditure, resulting in weight loss during the sleep deprivation period [8].

Sleep deprivation whether chronic or not can have huge impact on any economy globally leading to a great economic loss [9]. Pilcher and Huffcutt [10] reported that lack of sleep affects working memory, creativity, decision making, multitasking ability, response time, and focus. It was also reported that sleep deprivation prevents the brain from restoring its effectiveness, as it needs to work harder to accomplish the same amount of work [11].

Oxidative stress is known to be associated with several adverse outcomes such as cancers, neurological, cardiovascular and immunodeficiency diseases [12]. It was also reported to be involved in the mechanisms of aging, pathogenesis of cancer, atherosclerosis, diabetes, and neurodegenerative disorders [13]. Furthermore, SD in animals and obstructive sleep apnea syndrome in human was reported to be associated with increased oxidative stress [14].

Consequentially, free radicals been a product of oxidative stress were reported to accumulate during long wake period as a result of enhanced metabolic activity and are known to be responsible for the effects of sleep deprivation [15]. Thus, the free radical flux hypothesis proposed that the core function of sleep is to act as an antioxidant for the brain. Despite the appeal of this hypothesis, reported data to support it are conflicting. While some groups have reported decreased antioxidant capacity and oxidative damage in the brains of

sleep-deprived rats and mice [16], other reports have contradicted these findings [17].

An interesting consequence of the uncontrolled production of free radicals in the membrane of cell organelles was reported to be due to calcium (Ca^{2+}) leakage, which occurs concomitantly with the antioxidant enzyme release to cytosol. These events are believed to lead to cytotoxicity which may cause cell death by apoptosis or necrosis [4]. However, cells have a defense mechanism against the formation of ROS which includes production of antioxidant enzymes which include catalase (CAT), glutathione (GHS) and superoxide dismutase (SOD). Licorice (*Glycyrrizha glabra*) obtained from the dry roots and rhizomes of licorice plant have been demonstrated to be widely used in clinical prescriptions [18].

The pharmaceutical importance of licorice however lies in its capacity to yield a great variety of secondary substances, the most important bioactive compounds in licorice are triterpenes, flavonoids and polysaccharides [19]. These constituents have been shown to demonstrate biological activities including antitumor [20], antimicrobial [21], antiviral [22], anti-inflammatory [23], antidiabetic [24], immunoregulatory [25], antioxidant [26] hepatoprotective [27] and neuro-protective activities [28].

In a study to evaluate the antioxidant activity of licorice flavonoids, Liu et al [29] detected a significant change in the levels of oxidative stress markers in the colon of mouse, indicating that licorice flavonoids have strong antioxidant activities. However, there is no study till date that has investigated the effects of licorice on sleep deprivation and recovery in experimental animals.

Hence, the aim of this study was to demonstrate whether or not licorice root extract can regulate the imbalance between the reactive oxygen species (ROS) and production of antioxidant enzymes in the brain of sleep deprived rats.

METHODOLOGY:

Extract Preparation:

Licorice root powder was purchased from Amazon and was sold by Herbs and Crops Overseas, India with batch no: LRP-2017/02. To prepare the extract 50 g of the powder was mixed with 100 ml of sterile distilled water in a flask with occasional shaking for 10 minutes. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filters paper and kept in an airtight amber colored container. The dosage used in this present study was 150mg/kg body weight and was based on earlier study [30].

Animals:

Twenty - five 6-week-old male Wistar rats weighing averagely 200 ± 20 g purchased from Ekiti State University were used for the study. The rats were housed and maintained in

standard conditions of light, feeding and temperature in the Animal House of College of Medicine, Ekiti State University. The study was conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals [31]. Rats had unrestricted access to standard rat chow and tap water. After one week of acclimatization, the rats were randomly assigned to one of the following experimental groups (n = 5 per group) and treated accordingly. Rats Group I (Control) received distilled water (10ml/kg, orally) daily. Rats in Group II received distilled water (10ml/kg, orally) daily; designated as Sleep Deprived group (SD). Those in Group III received distilled water (10ml/kg, orally) daily; designated as Sleep Deprived, Sleep Recovery group (SD + SR). Those in Group IV received Licorice extract (150mg/kg, orally) daily; designated as Sleep Deprived with Licorice group (SD + Lic). Group V received Licorice extract (150mg/kg, orally) daily; designated as Sleep Deprived, Sleep Recovery with Licorice group (SD + SR + Lic).

Sleep models:

Sleep deprivation model was done in accordance with technique used by Oh et al [32]. The rats were sleep deprived for 5 days. The water in the tank was changed daily throughout the SD period.

For sleep recovery model, after 5 days of SD, the rats were given a SR period of 5 days. After the planned SR period, the rats were anaesthetized using a mixture of 25% (w/v) urethane and 1% (w/v) alpha chloralose (5ml/kg; Intra-peritoneal (i.p), BDH chemicals Ltd., Poole, England).

Determination of biochemical parameters:

Brain tissues were quickly excised and weighed. Thereafter, they were washed in cooled 0.15M NaCl and were then homogenized in 2 ml of ice-cold potassium phosphate buffer (0.1M, pH: 7.4) using an improvised homogenizer. Samples were centrifuged at 5000 rpm for 15 min to obtain the supernatant. The supernatant obtained were stored at -200C prior to use and later used to determine the various biochemical parameters.

Malondialdehyde (MDA): Bio-diagnostic: Diagnostic and Research Reagents. Lipid Peroxide (Malondialdehyde): Colorimetric method; Cat No MD 25 29; info@bio-diagnostic.com; www.bio-diagnostic .com.

Glutathione (GSH):

Bio-diagnostic: Diagnostic and Research Reagents. Glutathione reduced: Colorimetric method; Cat No GR 25 11; info@bio-diagnostic.com; www.bio-diagnostic .com.

Catalase (CAT): Bio-diagnostic: Diagnostic and Research Reagents. Catalase assay: Colorimetric method; Cat No CA 25 17; info@bio-diagnostic.com;

Statistical analysis:

Data are expressed as means ± standard error of the mean (SEM). Statistical group analysis was performed with Graph pad (Prism 7)

statistical software. Test of variance was done using ANOVA, followed by Tukey's multiple comparisons test. Statistically significant differences were accepted at $p < 0.05$.

RESULTS:

The result of the study showed increased change in brain weight among rats in group I, Group IV and Group V when compared with Group II as shown in table 1.

Result in table 2 showed significant increased changes in concentration of MDA among the rats in Groups II (51%), III (46.7%) and IV (31.3%) when compared with control. However, there was significant decreased change in concentration of MDA between Group IV (28.6%) and V (41.1%) when compared with Group II. Moreover, when compared with Group III, there was significant decreased change in concentration between Groups IV (22.4%) and V (36%).

For GSH activity as shown in table 3, our result demonstrated that rats in Group II (30.3%) and Group III (12.4%) showed significant decreased change in concentration when compared with control group. In furtherance, rats in Group III (20.5%), Group IV (24.6%) and Group V (30.8%) showed increased significant change in GSH concentration when compared with Group II. The concentration of GSH among rats in Group V was significantly higher than those in Group III (13%) and those in group IV (8.2%). Lastly in table 4, the result from our study showed significant decrease in CAT concentration among rats in Group II (69.3%) in Group III (45.4%) and Group IV (33.5%) when compared with the control group. The CAT concentration increased by 11.6% among rats in Group V compared to the control. The concentration of CAT among rats in Group II was significantly lower than those rats in Group III (43.8%), Group IV (53.8%) and Group V (72.9%). The CAT concentration among rats in Group V was significantly higher than those in Group 3 (51.8%) and those in Group IV (41.2%). These results clearly demonstrate that SD significantly affects the oxidative status of rats.

Table 1: Effect of licorice on brain weight in sleep deprived Wistar rat

Groups	Brain Weight (g)
Group I (Control)	1.76 ± 0.03
Group II (SD)	1.67 ± 0.01
Group III (SD + SR)	1.70 ± 0.03
Group IV (SD + Lic)	1.78 ± 0.03
Group V (SD + SR + Lic)	1.79 ± 0.02

Results are expressed as means ± S.E.M. of 5 rats per group

Table 2: Effect of licorice on Brain MDA in sleep deprived Wistar rat

Groups	Brain MDA (ng/mg protein)
Group I (Control)	92.16 ± 6.35
Group II (SD)	187.9 ± 8.66[a]
Group III (SD + SR)	172.9 ± 7.62[a]
Group IV (SD + Lic)	134.2 ± 5.52[a,b,c]
Group V (SD + SR + Lic)	110.7 ± 8.72[b,c]

Results are expressed as means ± S.E.M. n= 5.
[abc] p<0.05 vs Control, Group II and Group III.

Table 3: Effect of licorice on Brain GSH in sleep deprived Wistar rat

Groups	Brain GSH (µmole/g protein)
Group I (Control)	1.45 ± 0.03
Group II (SD)	1.01 ± 0.02[a]
Group III (SD + SR)	1.27 ± 0.03[a,b]
Group IV (SD + Lic)	1.34 ± 0.02[b]
Group V (SD + SR + Lic)	1.46 ± 0.02[b,c,d]

Results are expressed as means ± S.E.M. n= 5.
[abcd] p<0.05 vs Control, Group II, Group III and Group IV

Table 4: Effect of licorice on Brain CAT in sleep deprived Wistar rat

Groups	Brain Catalase (nmol of H2O2/min/mg protein)
Group I (Control)	0.821 ± 0.06
Group II (SD)	0.252 ± 0.04[a]
Group III (SD + SR)	0.448 ± 0.02[a,b]
Group IV (SD + Lic)	0.546 ± 0.05[a,b]
Group V (SD + SR + Lic)	0.929 ± 0.04[b,c,d]

Results are expressed as means ± S.E.M. n= 5.
[abcd] p<0.05 vs Control, Group II, Group III, Group IV

DISCUSSION:

SD is known to enhance metabolic rate which in turn increase oxidative stress even though sleep seems to limit metabolic requirements [33] and this is in tandem with our study. For instance, our study demonstrated significant increase in MDA level among rats in SD group (Group II) when compared with rats in other groups which is in consonant with previous study by Halliwell and Gutteridge [34] where MDA level was reported to be related to an increased level of lipid peroxidation in cell membranes. However, our results suggest that lipid peroxidation in certain regions of the brain following sleep deprivation can generate free radicals and this free radical can cause neuronal damage thereby affecting neuronal transmission at the synapse leading to neurological disorders. Although previous study by Gopalakrishna and colleagues reported there was no significant difference in the concentration of lipid peroxidation after sleep deprivation in Wistar rats [17]. However, rats in group IV and V had significantly lower MDA level when compared with those in Group III. This could be attributed to antioxidant property of licorice and compensatory mechanism of sleep which helps prevent the brain from oxidative stress by reducing neuronal metabolic activity with less oxygen consumption.

Furthermore, our study showed significantly reduced level of GSH among rats in SD group (Group II) when compared to control and other groups and this goes with previous study by D'Almeida et al [35] where GSH level was also shown to be reduced in the brain of Wistar rats during sleep deprivation. One of the mechanisms by which GSH induced its antioxidant effect is by generating oxidized glutathione (GSSG) [34]. Furthermore, Honda et al [36] also demonstrated that GSSG has an inhibitory action on the exciting synaptic membrane of rat brain. They also speculated that GSSG can promote sleep-enhancing activity which is due to its physiological modulation on glutamatergic neurotransmission in the brain.

Lastly in our study, the concentration of CAT among rats in Group II (SD) was significantly lower than those rats in Group III (43.8%), Group IV (53.8%) and Group V (72.9%).However, a number of studies reported that SD induced oxidative processes in several types of tissues, resulting in some cases, in cognitive impairment and behavioral changes [4]. For instance, Everson et al also showed in their study a reduction of 23% and 36% in liver CAT activity in rats that were sleep deprived [37].

In conclusion, the results of our present study showed that licorice has ameliorative effect on the imbalance between reactive oxygen species and production of antioxidant enzymes in the brain of sleep deprived rats.

Conflict of interest:

The authors declare no financial or other conflicts of interests in the design and interpretation of study results.

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ASSESSMENT OF SECONDARY METABOLITES AND ANTIMICROBIAL ACTIVITIES OF FOUR SOLVENT EXTRACTS OF *Vernonia amygdalina* LEAVES ON SOME SELECTED PATHOGENIC MICROORGANISMS

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Running title: Secondary metabolites and antimicrobial assay of *Vernonia amygdalina* leaves

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ABSTRACT:
Acetone, Hexane, Ethylacetate and Acetone / Hexane / Ethylacetate (50/30/20 v/v/v) extracts of *Vernonia amygdalina* leaves (Del, belonging to the family Asteraceae) were investigated for antimicrobial activities. Three different concentrations (100 mg/mL, 50 mg/mL and 25 mg/mL) of each extract were used against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25920 and *Candida albicans*. The disc diffusion method was used. Phytochemical screening and High Performance Liquid Chromatography (HPLC) analysis of constituents of the extracts were also carried out. Highest yield (14.25 %) after extraction was obtained for acetone extract followed by hexane extract (8.2 %), Acetone/Hexane/Ethylacetate extract (7.4 %) and ethylacetate (7.1 %) extract. Tannin, steroids, saponin, terpenoid, flavonoid, anthraquinones were present in each of the solvent extracts. Coumarin was present in both acetone and Acetone/Hexane/Ethylacetate extracts while glycosides were present only in the Acetone/Hexane/Ethylacetate extract. Protein was present in ethylacetate extract and absent in the other solvent extracts. The extracts revealed dose-dependent activities against all the test organisms. Highest range of inhibition zone (15.00 mm- 18.00 mm) was obtained for Acetone/Hexane/Ethylacetate extract at 25 mg/mL against the test organisms while lowest range of inhibition zone (7.00 mm- 10.00 mm) was obtained for acetone extract at 100 mg/mL. Various compounds (Oxalate, Phyrate, Epivernodalol, Vernodalol, Venonioside A, Vernonioside B, Vernodalin, Luteolin, Andrographolide, Andrographoside) were separated at different peak heights (mV) and at varying retention times from all the solvent extracts. The solvent extracts of *Vernonia amygdalina* can serve as good alternative to orthodox medicine in the treatment of infections associated with the three test organisms.

Keywords: *V. amygdalina*, Phyrate, Vernodalol, Pathogens, Antimicrobial activities, HPLC

INTRODUCTION:

The skin and mucous membrane are common routes of invasion of most pathogenic microorganisms, posing serious public health problems such as increased morbidity in patients that undergo surgical operations in developing countries [1,2], higher cost of health maintenance and even death. Some microorganisms associated with skin infections are Gram positive bacteria such as *Staphylococcus aureus* and Corynebacterium species, Gram negative bacteria such as *Escherichia coli* and *Enterobacter aerogenes* and yeast, *Candida albicans* [3].

Despite the vast production of a number of new antibiotics by pharmacological industries against pathogens, resistance among microorganisms has increased, probably due inappropriate use of the drugs resulting in the genetic ability of these microorganisms to transmit and adopt resistance to those drugs [4]. About 80 % of the populations in developing countries rely on medicinal plants [5,6] as alternative to orthodox medicine in their health care delivery system. In Nigeria, the majorities of citizens use medicinal plants and visit traditional medicine practitioners for their health care needs [7]. The greater interest in herbal medicine may be due to certain limitations of orthodox medicines in terms of high cost, limited availability, adulteration and associated toxicity [8].

Natural antimicrobials have been chosen from numerous medicinal plant parts (such as leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds). These parts are known to contain the highest concentration of active constituents which play important roles in bioactivity of medicinal plants and are used as new effective agents against drug resistance strains of microorganisms [9].

Medicinal herbs are commonly administered in many forms of preparations such as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician [10]. Some preparations are consumed in liquid form by the patient, as a tisane or a (possibly diluted) plant extract [11]. Determination of biologically active constituents from medicinal plants largely depend on plant part used as starting material, solvent used for extraction and extraction procedure [12]. Properties of a good solvent in plant extractions include, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate [13] . Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds. A study reported that extraction of tannins and other phenolics was better in

aqueous-acetone than in aqueous-methanol [10,14]. Both acetone and methanol were found to extract saponins which have antimicrobial activity [12].

Vernonia amygdalina Del, commonly called bitter leaf, belongs to the family Asteraceae. It is a perennial plant predominantly found in Africa. It is known in different parts of Nigeria as '*Ewuro'* among the Yorubas, '*Onugbu'* among the Igbos and '*Chusar-doki*' or '*fatefate'* among the Hausas. Many Nigerian households attribute many folkloric uses to the leaves of *V. amygdalina*, including culinary herb in soup, as food vegetable, digestive tonic and as appetizer. Hausa females of Nigeria also consume *V. amygdalina* infusion to enhance sexual adequacies. A number of scientific findings have been reported on *V. amygdalina*. The plant had been reported to possess antimalarial, antimicrobial, antidiabetic, laxative, febrifuge, antihelminth properties [15]. The leaves have also been reported to have wound healing properties [16]. Oyugi *et al*. [17] showed that fractions of hexane, chloroform and n-butanol extracts of *V. amygdalina* inhibited the growth of human breast cancer cells even at concentrations of 25 mg/mL.

Despite the available information on *V. amygdalina*, the search for more information on medicinal plants has continued unabated. In addition, it is important to know that the type of solvent used in extraction of plants affect the type and amount of constituents detected in their extracts. Hence, the present study aimed at using different solvents with varying polarities to extract *V. amygdalina* leaves, determining the antimicrobial activities of the different solvent extracts of the plant, screening the different solvent extracts for secondary metabolites using preliminary phytochemical screening method and HPLC method to separate the active components in the solvent extracts.

METHODOLOGY:

Plant materials:

Fresh leaves of *Vernonia amygdalina* were harvested in Adewole area, Ilorin, Kwara State, Nigeria. They were authenticated at Herbarium Unit of Department of Plant Biology, University of Ilorin, where a voucher specimen was deposited (UILH/007/972).

Preparation of the crude extracts:

The leaves were carefully washed under running water and distilled water to get rid of dust particles and sand. They were completely dried in aerated place for 3-4 days and pulverized into fine powder using electric miller (Master Chef Blender, Mode MC-BL 1980, China). Crude extract of the leaves was obtained by soaking the powdered leaves in different solvents (1:10 w/v of plant material to solvent) of varying polarities; acetone (most polar), hexane (medium polar), ethyl acetate (least polar) and a combination of the three solvents (acetone/ hexane/ ethylacetate 5:3:2 v/v/v). The extraction was done for 48 hours at

room temperature with shaking at 150 rpm [18]. The homogenate obtained was subsequently filtered through Whatman No. 1 filter paper [19] and the filtrate was evaporated to dryness using rotary evaporator (Model RE Zhengzhou, Henan China). The concentrated extracts were then collected, weighed, packed in sterile air tight containers and labeled. Percentage yield of the extract was calculated and recorded. They were kept in the refrigerator at 4 ºC until needed for analysis.

Preparation of samples for HPLC:

Powdered dried leaves (1.0 g) of *V. amygdalina* were macerated with acetontrite/water (1:1; v/v, 10.0 mL), then centrifuged at 3000 rpm for 10 minutes. The material was filtered and the crude extract obtained was analyzed directly by HPLC-UV.

Sterility testing:

This was carried out using a modified method of Sule and Agbabiaka [20]. Serial dilution of 1.0 g of each extract was made to reduce the concentration. A quantity (2 ml) of each extract was inoculated into 10 mL of Muller Hinton broth, incubated at 37 ⁰ C for 24 hours. The absence of turbidity or clearness of the broth indicated sterility of the extract.

Preparation of stock solution of the extracts:

Diluent of 5.0 % Dimethylsulphoxide (DMSO) was prepared by adding 95 mL of water to 5 mL of DMSO. Stock solution of the extract was prepared by dissolving 2.0 g of extract into sterile test tube containing 20.0 mL of 5.0 % DMSO. The test tube was labeled as 100.0 mg/mL from which other working concentrations (50.0 mg/mL and 25.0 mg/mL) were prepared.

Preparation and Impregnation of discs:

Paper discs were prepared from good quality filter paper (What man No. 1). The discs were autoclaved for 15 minutes at 15 lbs pressure and allowed to cool. About 5 pieces of the sterilized paper discs were arranged using sterile forceps in sterile petri-dishes. Sterilized discs were aseptically impregnated with approximately 20 µl of 100 mg/mL concentrations of each solvent extract using mechanical pipettes. More sterilized discs were also aseptically impregnated with approximately 20 µl of other concentrations (50 mg/mL and 25 mg/mL) respectively. All impregnated discs were allowed to fully dry in incubator at 45 ⁰ C for 18-24 hours and were stored in labeled airtight containers at 20 ° C till they were ready for use [21]. The standard antibiotic discs used as positive control were Chloramphenicol and Gentamycin (Sigma, Saint Louis, MO, USA) for the bacterial strains and griseofulvin (Bristol, New York, USA) for yeast.

Test Organisms:

Three isolates used in this study were *Staphylococcus aureus* ATCC 25923, a Gram-

positive organism, *Escherichia coli* ATCC 25920, a Gram-negative Organism and a yeast, *Candida albicans*. They were obtained from the Microbiology Laboratory of University of Ilorin Teaching Hospital, maintained on Nutrient agar slant for the bacteria and Sabouraud Dextrose Agar slant for the yeast. They were cultured and sub-cultured to check for their purity, then their morphology and Gram staining was carried out to confirm their identity. The inocula were standardized using McFarland standard [22].

Preparation of McFarland turbidity standard:

This was prepared by mixing 99.5 ml of 1% sulphuric acid and 0.5 ml of 1.175% w/v barium chloride ($BaCl_2.H_2O$). The mixture was dispensed in 3-4 ml amount in tube.

Standardization of Inocula:

Colonies from microbial culture (3-5 colonies) was aseptically suspended into 5 mL of Mueller-Hinton Broth (MHB) using a sterile loop and incubated at 37 °C for 18 hours and yeast into Sabouraud Dextrose Broth (SDB) at 30 °C incubation period. The microbial growth were harvested using the same broth medium as used earlier (0.1 mL of the innoculum into 19.9 mL of the broth), the absorbance was adjusted at 580 µm and diluted to 0.5 McFarland turbidity equivalence (1.5×10^8 CFU/ mL) to obtain equivalence of 2×10^6 CFU/ mL for bacteria and 2×10^5 spore / mL for the yeast. This was used within 20 minutes of standardization [23].

Antimicrobial activities of the solvent extracts:

Antimicrobial activity of each solvent extract was tested using a modified disc diffusion method described by Ncube *et al*. [12]; a sterile cotton swab was dipped into the standardized *Escherichia coli* ATCC 25920 inoculums, pressed against the inside wall of the test tube to remove excess fluid and streaked uniformly on the surface of the solidified 20 mL Mueller-Hinton agar (MHA; Becton-Dickson, USA) for bacteria and Sabouraud Dextrose agar (SDA) for the yeast. The streaking was carefully done three times. The plates were rotated about 60 degrees to ensure even distribution of the inoculums on the agar surface, then allowed to dry for 5 min. Sterile filter paper discs loaded with the plant extract (100 mg/mL concentration) was placed on top of Mueller-Hilton agar plate (90 mm plate) using sterile forceps, the discs were pressed gently to ensure uniform contact with the agar surface. This was allowed to stand on the bench for 30 minutes for proper diffusion of extract from the disc into the inoculated medium and thereafter incubated at 37 °C for 24 hours for bacteria and at 30 ° C for 48 hours for *C. albicans*. This was repeated for *Staphylococcus aureus ATCC 25923* and for other concentrations (50 mg/mL and 25 mg/mL respectively). Control standard discs of chloramphenicol, gentamicine and griseofulvin for bacteria and yeast were set up. The diameters of zones of inhibition around the

discs were measured using a Vernier caliper (mm) and recorded. This study on the extracts and controls were carried out in triplicates to ensure reliability and the average of three replicates was calculated and recorded.

HPLC analysis:

A modular Shimadzu (Nexeramx) LC-10 system comprised of a LC-10AD pump, a CTO-10A column oven, a SPD-10A UV-DAD detector, a CBM-10A interface and a LC-10 Workstation was used. A LC-18 column (250 mm x 4.6 mm ID x 5 mm) from (Ubondapak , Bellefonte, USA) was employed at 30 ° C. Separations were done in the isocratic mode, using acetonitrile: water (40:60; v/v) at a flow rate of 1.0 mL per minute with an injection volume ("loop") of 10 µL, UV detection was at 254 nm.

RESULTS:

The percentage yield with the different solvents and the physical properties of the solvent extracts after evaporation are presented in Table 1. The *In vitro* antimicrobial assay result of different concentrations of the various solvent extracts against the test organisms is represented in Figure 1. A total of nine phytochemical constituents (Tannin, steroid, saponin, terpenoid, flavonoid, anthraquinones, protein, glycosides and coumarins) were screened for. Table 2 shows the constituents extracted by the four solvents used. The components separated by HPLC of the four solvent extracts of *V. amygdalina* leaves were visualized in the form of peaks in chromatograms. The results of the retention time and peak heights of the various components are presented in Table 3.

Table 1. Percentage yield of *V. amygdalina* Leaves after Evaporation

Solvents	Dry raw powder of plant (g)	Plant extract yield from dry raw powder (g)	Plant extract percentage yield (%)	Color
Acetone	200	28.5	14.25	Green
Hexane	200	16.4	8.2	Green
Ethylacetate	200	14.2	7.1	Green
Acetone / Hexane / Ethylacetate	200	14.8	7.4	Brown

Fig.1. In vitro Antimicrobial Effects of Different Concentrations of Various Solvent Extracts of *V. amygdalina* leaves against some Selected Pathogens

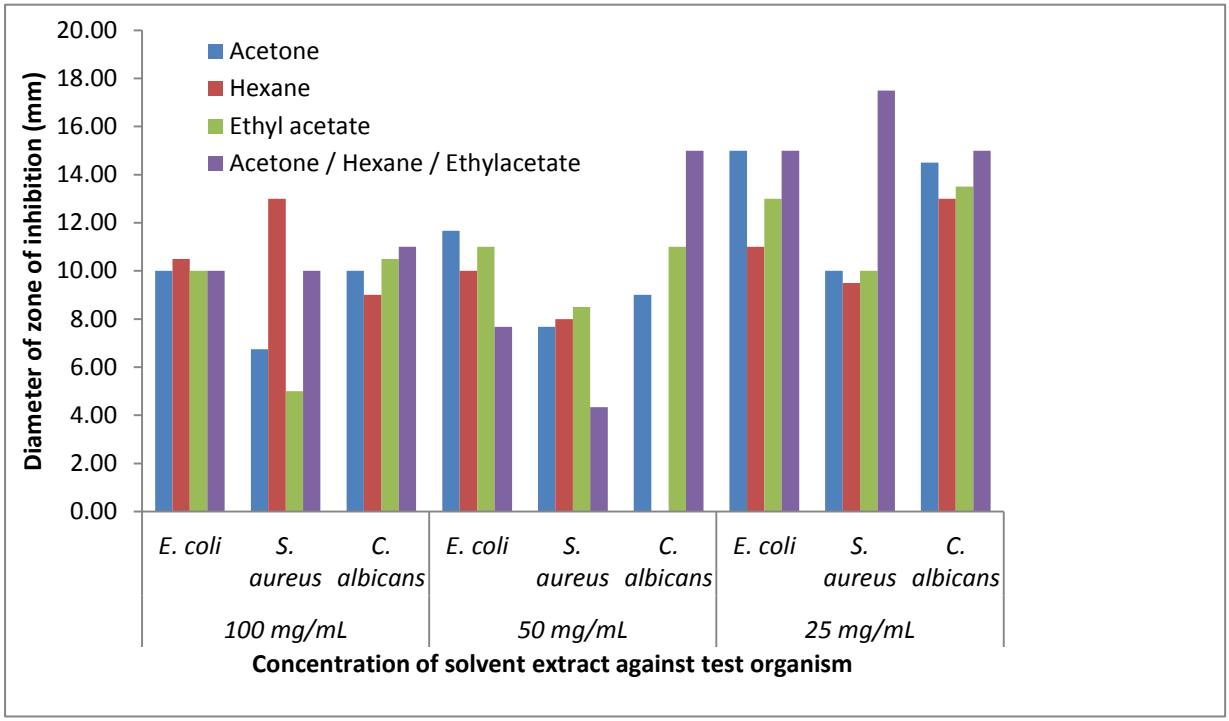

Table 2. Phytochemical constituents of four solvent extracts of *V. amygdalina* leaves

Composition	Acetone	Hexane	Ethylacetate	Acetone / Hexane / Ethylacetate
Tannin	+	+	+	+
Steroid	+	+	+	+
Saponin	+	+	+	+
Glycosides	–	–	–	+
Terpenoid	+	+	+	+
Protein	–	–	+	–
Flavonoid	+	+	+	+
Anthraquinones	+	+	+	+
Coumarins	+	–	–	+

Key: + = Present; – = Absent

Table 3. Retention time and peak heights in the different solvent extracts of *V. amygdalina* leaves separated at different intervals by HPLC method

COMPONENTS	Acetone		Ethylacetate		Hexane		Acetone / Hexane / Ethylacetate	
	Retention (Mins)	Peak height (mV)	Retention (Mins)	Peak height (mV)	Retention (Mins)	Peak height (mV)	Retention (Mins)	PEAK HEIGHT (mV)
OXALATE	1.266	34.054	1.266	33.477	1.267	33.348	1.266	32.751
PHYRATE	2.516	24.219	2.516	22.367	2.516	21.953	2.516	20.039
EPIVERNODALOL	2.750	24.994	2.750	22.904	2.750	22.437	2.750	20.278
VERNODALOL	4.450	11.959	4.450	8.136	4.450	7.281	4.450	3.333
VERNONIOSIDE A	4.700	11.786	4.700	7.707	4.700	6.796	*	*
VERNONIOSIDE B	5.466	6.921	5.466	2.215	*	*	*	*
VERNODALIN	11.050	158.213	11.050	153.998	12.166	47.515	11.050	149.459
LUTEOLIN	12.166	62.440	12.166	54.557	12.166	62.440	12.166	45.596
ANDROGRAPHOLIDE	13.700	38.239	13.700	26.675	13.700	16.345	13.700	9.278
ANDROGRAPHOSIDE	17.616	5.504	17.616	4.264	17.616	1.766	17.533	1.489

*= absent

DISCUSSION:

Findings in this study revealed that highest yield was obtained for acetone extract more than other solvent extracts. This is in accordance with the report of Sengel *et al*. [24] who reported that acetone was effective at extracting more phytoconstituents from plants than other solvents.

Tannin, steroid, saponin, terpenoid, protein, flavonoid, anthraquinones, glycosides and coumarins detected in the different solvent extracts in this work have been reported to play significant roles in bioactivity of medicinal plants since medicinal values of plant lie in these phytochemical compounds which produced a definite and specific action on the human body [25]. This result is also in line with the study conducted by Imaga *et al*. [26] who detected the presence of flavonoid, tannins, saponins, glycosides and terpenoids as the most predominant constituent in *Vernonia amygdalina* leaves extracts. Secondary metabolites (tannins, saponins, steroids, flavonoids, steroid, glycoside, anthraquinones, terpenoids) are suspected to be responsible for the bitter taste of this plant and they exert antimicrobial activity through different mechanism [27]. Flavonoids constituent exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, antiangionic, analgesic, anti-allergic, cystostatic and antioxidant properties, anticancer activities [28, 29]. Hence, the compounds detected in the extracts may be responsible for the antimicrobial activities presented by zones of

inhibition observed against pathogenic microorganisms in this work.

Previous reports are on extraction of phenolic compounds typically from plant material using solvents as methanol, ethanol, acetone and ethyl acetate [30,31]. However, in the present study, the four solvents used for extraction of compounds in *V. amygdalina* leaves have been shown to affect the quantity and quality of the components separated by HPLC. This agrees with several studies that characterized bioactive compounds from *V. amygdalina* as flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones [32]. In conformity with compounds separated in this work are the reports of Sobrinho *et al*. [33] and Erasto *et al*. [34] who variously revealed that the main bioactive constituents of the leaves of *V. amygdalina* are sesquiterpene lactones, vernonioside A1, vernonioside A2, vernonioside B1, vernonioside B2, vernodalin, vernolepin, vernomygdin, vernodalol, and vernodalinol. Oxalate, phytate, luteolin, andrographolide and andrographoside were detected in this study but were not listed in the reports given by those researchers.

Constituents were separated at sharper height peaks from acetone extracts more than from other solvents extracts. This is in concurrence with the study conducted by Michiels *et al*. [35] where acetone-based mixtures were also more effective solvents than the methanol-based mixtures for phenolic extraction yields from fruits and vegetables. However, in the report of Tomsone *et al*. [36], the best solvents for phenolic extraction from horseradish roots were ethanol and ethanol/water solutions [36].

Vernodalol, Vernonioside A, Vernonioside B and Vernodalin separated in the different solvent extracts of *V. amygdalina* leaves in this study justified their antimicrobial activities as presented by zones of inhibition obtained against the test organisms. Amodu *et al*. [37] and Luo *et al*. [38] variously reported that sesquiterpene lactones (vernodalinol, vernolepin, vernomygdin, hydroxyvernolide, vernolide and vernodalol) possessed antitumoral and antimicrobial properties, and exhibited significant bactericidal activity against bacteria. Isolated vernoniosides from *Vernonia amygdalina* leaves exhibited anti-inflammatory property and was used in the treatment of gastrointestinal disorder when tested with murine macrophage cell line and wild chimpanzees, respectively [39]. Presence of phenolic compounds in plants has been reported to play important role in shaping the biological properties of plants including antioxidant and antimicrobial properties [40].

All the solvent extracts of *Vernonia amygdalina* leaves showed varying concentration dependent activities against the test pathogens. Gill and Holley [41] reported that antimicrobial components (terpenoid, alkaloid and phenolic compounds) of plant extracts

interact with enzymes and proteins of cell membrane of microorganisms causing its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for amino acids biosynthesis. Findings in this study agrees with the findings of Owu *et al*. [42] who also reported dose-dependent contraction of ileums of guinea pigs induced with gastric secretion from aqueous extracts of *V. amygdalina*. However, lowest concentration (25 mg/mL) of all the solvent extracts showed higher activities with wider zones of inhibition against the tested pathogens than other concentrations (50 mg/mL and 100 mg/ mL), this may imply that the plant is highly potent even at reduced dosage.

Although, zones of inhibition obtained by the plant extracts was not as high as what was produced by the commercial drugs. The small molecular size possessed by antibiotics aids their solubility in diluents as this could enhance their penetration through the cell wall into the cytoplasm of the organism. Doughari *et al*. [43] reported that since antibiotics is in a refined state and plant extract in crude state, a higher antimicrobial activity may be recorded for commercial drugs.

CONCLUSION:

It could be deduced from this study that *V. amygdalina* leaves contain active constituents of pharmacological significance and extracts from the plant should be consumed at much reduced concentration so as to have a better treatment margin and reduced risk of dosage induced-toxicity. Results presented in this study also indicates that HPLC is a useful tool for assessment of components of different solvent extracts of plants, components which if properly screened could be harvested and used in the development of new drugs.

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ASSESSMENT OF PATIENTS' KNOWLEDGE TO THEIR DISPENSED MEDICATIONS AT PORT MORESBY GENERAL HOSPITAL, PAPUA NEW GUINEA

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ABSTRACT:
Limited information on drug use indicates that medicines are not optimally used. Inadequate knowledge of medication uses may lead to overuse of medicines or patient non-compliance with a medicine regimen, and result in serious outcomes. The aim of this study was to assess the patients' knowledge on dispensed medications in pharmacy at Port Moresby General Hospital (PMGH). This was a descriptive cross-sectional study utilizing a self-administered questionnaire adapted from the WHO "Guide to Good Prescribing Practical Manual". The questionnaire sought to elicit major classes of medicines prescribed, knowledge of patients on indications, how to use the medicines, precautions and possible adverse events. A total of 130 patients from PMGH participated in this study. Although majority of patients (94.6%) knew the instructions on the use of the dispensed medicines in terms of route of administration, dosage and frequency of usage; only 74.6% of them knew the duration of medicines use. Further, 19.2% of participants did not know the indications for which their medicines were prescribed. Knowledge on adverse effects was also significantly low i.e 34.7%. Lack of understanding of warnings and precautions was rated at 42.3%. These figures may contribute to poor patient compliance and cause possible harm to the patients. The results demonstrated lack of adequate information given to patients on their prescribed medicines. It is recommended that prescribers be re-trained on the importance of giving adequate information on medications given to patients, particularly to patients with low educational background.

Keywords: Patients' Knowledge, prescribed medicines, WHO guideline, PMGH, Papua New Guinea

INTRODUCTION:

Patients need information and adequate instructions to accept and follow the treatment and to acquire the necessary skills to take the drugs appropriately [1]. Unfortunately, researchers found that most patients do not understand their diseases or the consequences of not adhering to a medication regime [2]. Data show that in 2002 more than 50% of all medicines prescribed worldwide were dispensed inappropriately, while 50% of the patients failed to take them correctly [3]. Poor understanding of instructions inhibits a patient's ability to adhere to therapy as prescribed [4].

Findings on patient knowledge of medicines dispensed in developing countries are diverse, probably because the number of dispensing attributes recorded and literacy levels varied from study to study [5]. In a study in Ghana, 59% of the patients did not know what their conditions were, 81.8% did not know the right dosing schedule, while only 41% of the patients had received information about their conditions. This was despite the large number of patients who had expressed interest in getting information about their medicines.

Similar study in India by Singh J et al [7], a considerable number of patients were reported to be aware of the therapeutic effects of the drugs and the manner of administration; but the patients lacked information regarding the side effects, and warnings on overdose. In addition, a study in Brazil by Samant Frohlich et al reported the highest levels of patients' knowledge in drug administration frequency, therapeutic indication and duration of treatment. The lowest level of knowledge was in drug dosage, adverse effects and what to do when one or more drug doses were missed [8]. In a related study in South Africa by Ramathuba in 2008, it was evident that although more than 70% of interviewed patients took their medications correctly, almost 80% had not been warned of the side effects of the medicines [6].

Lack of knowledge on side effects would potentially affect medication compliance and medication safety particularly in older patients with chronic diseases, and more likely to be taking multiple medications [10].

Other studies have revealed patients' increased desire for information on medication risks, adverse effects, and on medical conditions being treated [11-12]. A limited study carried out at PMGH in Papua New Guinea (PNG) reported out of 21 patients only 2 (9.5%) knew what to expect therapeutically from their medications. How the medicines would alter the disease process, possible side effects and what to do when these occur and any possible interactions with other prescribed and non-prescribed medications, including impacts of alcohol were not discussed with the patients [13]. While a number of similar studies have recently been conducted overseas, there has been insufficient published information in PNG.

The PNG National Medicines Policy aims to make available to the public and health professionals, practical information on medicines, including instructions for their proper usage in order to achieve patient adherence to treatments and consequently good therapeutic outcomes [14]. Anecdotal evidence suggests that medicines are often given to patients with little or no verbal instructions. Seemingly, the pharmacist assumes that the patient has already received adequate instructions from the prescriber and has understood it. Currently there is no published data on assessment of patients' knowledge on the prescribed medicine at an outpatient level, particularly in PMGH, a major referral hospital in the country.

The aim of this study was to assess the patients' knowledge on dispensed medications in pharmacy at Port Moresby General Hospital.
The objectives of the study are to use the data obtained to assess patient's knowledge on the precaution and warnings of the medicine, and their knowledge on adverse effects of the medicine. In addition, to determine the patients' primary source of obtaining information on prescribed medicines.

METHODOLOGY:

The present study was conducted in PMGH, which is the major referral, specialist and teaching hospital in PNG. It is located in the National Capital District (NCD), PNG. The staff members included leading physicians and surgeons in PNG and specialists in all areas in medicine. There are eight pharmacists (4 full time and 4 interns) and two pharmacy technicians in the pharmacy department. On average, 200-300 prescriptions are received and processed daily.

This was a descriptive cross-sectional study that utilized questionnaire for data collection. The questionnaire was adopted from the WHO "Guide to Good prescribing - A Practical Manual", with some modifications.[15]. The answers to questions were either "Yes" or "No". Yes, means the patient was aware of the required information, while No, indicated that the patient did not know the information requested. The questionnaire was pre-tested on 15 patients in the first week of data collection and thereafter, refinements were made. This research was approved by the Research and Ethics Committee of School of Medicine and Health Sciences, and also approved by the Director of Medical Services PMGH. Data was collected over a period of two weeks. Patients who had collected medicine from the outpatient pharmacy and those who were older than 19 years of age were included in the study. Using a linear systematic random sampling procedure, every third patient leaving the hospital pharmacy was approached and asked to participate in the study. Also included were patients prescribed one of the following; Anti-Diabetics, Anti-Hypertensive's, Anti-epileptics, Anti-parkinsonism, Anti-psychotic,

Eye preparations, Inhalers, Macrolides (Erythromycin),Nitroimidazoles (Metronidazole), H2 Antagonist (Ranitidine/Cimetidine), and Non-Steroidal Anti-inflammatory Drugs (NSAID). These target drugs were chosen as they required definite information on usage, side effects, important interactions or entailed complex regimens or techniques for use. If a patient did not fulfill the above criteria the next one on the row was considered. The pharmacists were aware of the study in progress but to avoid bias, they were not shown the questionnaire.

Exclusions: Patients were excluded if they were medical or nursing staff, if they could not communicate, and if they refused to participate. If more than one medicine was prescribed, only the first one on the row was selected.

The nature and scope of the study was explained to the participants and those that consented were given the questionnaire. The patients answered the questions themselves. In the case of illiterate patient the questions were read and explained to the patients in tok-pisin language and answers were recorded in English.

Data were tabulated in Excel spreadsheets. Chi square tests were used; p values were calculated to determine the significance ($p< 0.05$).

RESULTS:

A total of 130 patients consented to participate in the study. The demographic characteristics of the patients are presented in Table 1. A chi-square test of independence was performed to examine the relation between gender and the age groups (2x5 tables). There were no significant differences between males and females among the age groups who participated in the study; ($X2 = 3.1387$; $p=0.5349$ at $p< 0.05$). Majority (74.6%) were within the age of 40 years and above. Furthermore, most of the participants had primary (37.7%) or high school education (50%), while 12.3% had no formal education.

Of the 130 patients 80 (61.5%) were provided information by the prescribing health professional (doctor or nurse). The fact that a significant proportion of patients (28.5%) obtained information from the product package labels pose a huge challenge for the patients literacy needs. In this study 23% (16/130) of the patients had no formal education and would need medicines information from the prescriber or pharmacist. Apparently, the dispensing pharmacists provided staggering low information to a proportion of only 6.2% (8/130) of the patients. Such a low pharmacist participation could be explained by the high workload of a small number of pharmacists (4 permanent and 4 interns) engaged in the largest referral hospital, where they process between 200 – 300 prescriptions per day.

Table 2 outlines the therapeutic classes of medicines encountered in the study. Prevalence of life saving medicines such as anti-hypertensives, treatments for diabetes and

for various psychoses has been highlighted as categories of medicines on which special attention must be paid. Other results about the patients' knowledge on a number of medicines' events and medication requirements constitute the core of this study and the findings are depicted in Tables 3 and 4. Table 5 highlights sources of medicines information.

Table 1: Demographic characteristics of the patients (n=130)

Age Category	**Total (%)**	**Males (%)**	**Females (%)**
Total	130 (100)	62 (47.7)	68(52.3)
19-29	15 (11.5)	7 (5.4)	8 (6.2)
30-39	18 (13.8)	7 (5.4)	11 (8.5)
40-49	20 (15.4)	13 (10.0)	7 (5.4)
50-59	27 (20.8)	12 (9.2)	15 (11.5)
>60	50 (38.5)	23 (17.7)	27 (20.8)
(Chi-square = 3.1387; p=0.5349; result not significant at p< 0.05).			

Education	**Total (%)**	**Males (%)**	**Females (%)**
Primary/High school	49 (37.7)	23 (17.7)	26 (20.0)
Secondary Ed. & above	65 (50.0)	37 (28.5)	28 (21.5)
No formal Education	16 (12.3)	6 (4.6)	10 (7.7)
Total	130 (100)	66 (50.8)	64 (49.2)
Chi- square = 2.3996 ; p = 0.3013 result not significant at p < 0.05)			

Occupation	**Total (%)**	**Males (%)**	**Females (%)**
	130 (100)	66 (50.8)	64 (49.2)
Student	5 (3.9)	2 (1.5)	3 (2.3)
Unemployed	39 (30.0)	17 (13.1)	22 (16.9)
Employed	28 (21.5)	19 (14.6)	9 (6.9)
Retired	43 (33.1)	25 (19.2)	18 (13.9)
Subsistence Farmer	15 (11.5)	3 (2.3)	12 (9.2)
	130 (100)	66 (50.8)	64 (48.2)
Chi-square = 10.9238; p = 0.027434: results are significant at p < 0.05.).			

Table 2: Frequency of therapeutic classes of medicines prescribed based on number of patients at Port Moresby General Hospital

Class of Medicine	N (%)
Anti-hypertensives*	39* (30.0)*
NSAID	21 (16.2)
Anti-Diabetic*	15* (11.5)*
Nitroimidazoles	14 (10.8)
Anti- Psychotics*	11* (8.5)*
Inhalers	8 (6.2)
Macrolide	7 (5.4)
Eye Preparations	4 (3.1)
H2 Antagonists	4 (3.1)
Anti-Parkinson's	4 (3.1)
Anti-Epileptic	3 (2.3)
	130 (100)

Figures in parenthesis are percentages * Lifesaving medicines

Table 3. Participants' Knowledge on Medication Instructions

	Male	**Female**	**Total**	p - value
	N (%)	N (%)	N (%)	
Knew the amount to take, route & frequency				
Yes	59 (45.5)	63 (48.5)	122 (93.9)	0.0319
No	7 (5.4)	1 (0.8)	8 (6.1)	X2=4.6015
Knew the duration of the medication & when to stop				
Yes	51 (39.2)	46 (35.4)	97 (74.6)	p=0.4796
No	15 (11.5)	18 (13.6)	33 (25.4)	X2=0.4998

Table 4: Knowledge of participants on indicated uses, effects and precautions of Medicines at PMGH (N=130)

	Males	**Females**	**Total**	p-value
	N (%)	N (%)	N (%)	
Knew the indication of the medicine				
Yes	52 (40)	53 (40.8)	105 (80.8)	p = 0.5605;
No	14 (10.7)	11 (8.5)	25 (19.2)	X2=0.3388
Knew what to do when dosage missed / forgotten				
Yes	32 (24.6)	36 (27.8)	68 (52.3)	P=0.3755
No	34 (26.2)	28 (21.5)	62 (47.7)	X2=0.7854
Understood precautions or warnings of the medicine				
Yes	12 (9.2)	63 (48.5)	75 (57.7)	p< 0.05
No	54 (41.5)	1 (0.8)	55 (42.3)	X2=82.49
Knew adverse Effects of the medicines				
Yes	24 (18.5)	21 (16.2)	45 (34.6)	p=0.6705
No	42 (32.3)	43 (33.1)	85 (65.4)	X2=0.181

Table 5: Patients sources of medication information

Sources	**N (%)**
Prescribers (doctors & nurses)	80 (61.4)
Label on Medicine Package	37 (28.5)
Relatives/Friends	5 (3.9)
Pharmacists & intern pharmacists (4+4)	8 (6.2)
Total sources	130

DISCUSSIONS:

As shown in Table 1, almost equal numbers of males and females participated in this study. It was noted that majority of the respondents were 40 years and above (75%), retired (43%), and were on recurrent treatments. Thus, they were supposedly more familiar with their medications. Most remarkable is the relatively larger proportion of unemployed women 22 (16.9%) and relatively larger proportion of women working in the informal sector as subsistence farmers (n=12 (9.2%) against men (n=3 (2.3%). These unfavorable indications are a reflection of low participation of women in the socioeconomic sectors of the country and

related gender inequalities, which must be addressed separately by PNG government.

Knowledge on Medication Instructions:

As in Table 3, a fairly high proportion of the patients were well aware of the route of administration, and dosage schedule (93.9%). These results were similar to the findings of similar study by Singh et al., where knowledge on dose and on frequency of administration was found to be 85.4% and 86.4% respectively for dispensed medicines [7]. In our study most patients were aware of the timing (93.9%) and the duration (74.6%) of the treatment regimens, given that they had taken the medicines before. These basic findings are consistent with knowledge related factors that affect patient adherence to medication regimens as pointed out by Antonia Kalogianni [17], and Beata Jankowska-Polanska et al [18]. In Table 4, of the 130 patients in the study, 105 (80.8%) knew the indications of their medicines. However, only 45 (34.7%) of them knew about important adverse effects of the medicines ($X2 = 0.181$; $p = 0.6705$). Furthermore, 68 (52.3%) of them knew what to do when dosage is inadvertently missed. Knowledge on the therapeutic indications and on adverse effects enables patients to assume adherence to medication as an important part in disease control and treatment.

These findings are in similar pattern to results of a study carried out by Samanth Frohlich et al. in Brazil although the latter scenario indicated even much lower proportion of 23.3% patients who did not know what to do when a dose is missed or forgotten [8]. Undoubtedly, lack of information is one of the factors contributing to treatment failure; hence the findings of this study underscore the need and provision for such information to patients as stressed by Antonia Kalogianni [18] and on the study by Beata Jankowska-Polanska et al where knowledge on hypertension was found to be a significant determinant of good adherence [19].

Knowledge on Precautions and Warnings:

Overall, only 75 (57.7%) of the 130 participants were aware of the warnings and precautions linked with the prescribed medications, while 55 (42.3%) were not. Despite the low educational level, relatively more women 63 (48.5%) than men 12 (9.2%) knew the precautions and warnings, and the difference was significant ($X2=82.49$ Yates corrected; $p < 0.05$), demonstrating a reassuring scenario, where women would in most circumstances be the ones expected to administer medicines to children or help minors with their medications.

A high proportion of the participants; 55 (42.3%) exhibited no knowledge of the precautions and warnings. Although our study demonstrated a relatively higher (57.7%) knowledge than a similar study by Samant Frohlich et al in Brazil [8] with only 31% knowledge about the precautions and warnings; it suggests the need for increased

information and awareness to patients and clients in order to improve adherence to medication. Further assessment of medicines therapeutic classes in Table 2 revealed that categories of medicines prescribed were mostly lifesaving medicines such as: anti-hypertensives, anti-diabetics, antipsychotics, anti-epileptics, which must be used continuously to achieve desired therapeutic actions with lesser complications. It could therefore be suggested that patient education and information would increase the knowledge and awareness needed to improve adherence with treatment regimens, hence improving self-care and quality of life. In addition, possible adverse drug reactions (ADRs) and drug-drug or food-drug interactions could actually be prevented by improvements through patients' medication information and counseling. In this study, patients (65.4%) appear to be most uninformed about the adverse effects of their medicines.

The high percentage of "No" knowledge of adverse effects is likely due to the lack of provision of information by the prescribers and other health professionals, such as the pharmacist. Possible reasons for the lack of information regarding adverse effects may be due to the fear of alarming the patients. The results in the present study corroborates well with results of other studies where deficiencies in knowledge of adverse effects were documented [2, 3, 4].

CONCLUSIONS:

The percent of patients who knew the correct dosage of their medication was very low. The dispensing time and dispensing counseling time was very short to give adequate drug information for the patient. Highest levels of knowledge were observed in route of administration, dosage of the medicine, schedule of administration. Considerable numbers of participants were aware of the duration of the treatment whilst lowest levels of medication knowledge occurred about adverse effects, what to do when one or more doses are missed and about the precautions and warnings. In addition, the prescribers, package label and information leaflet were the two main sources of patients' knowledge on medicine's actions.

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PROVIDERS' PERCEPTIONS OF THE KEY ISSUES AFFECTING THE DELIVERY OF QUALITY SERVICES AT A DENTAL CLINIC IN PORT MORESBY, PAPUA NEW GUINEA: A QUALITATIVE DESCRIPTIVE STUDY

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ABSTRACT:
The aim of this study was to identify factors that affect the dental service delivery in a dental clinic from the context of Papua New Guinean dental providers and to make practical recommendations to improve dental service delivery. This qualitative descriptive study used individual interviews with seven dental providers to identify the factors that were affecting quality dental service delivery in a dental clinic in Papua New Guinea. The factors influencing the quality of dental service fell into three categories and 8 themes. The categories were environmental factors with themes of resources and facilities, leadership and management, partnership development and collaboration; provider-related factors with themes provider competence, provider motivation and satisfaction; and required measures with the themes of a new dental clinic building, renovation of the main clinic area and organizational structure. Quality dental service is achieved when there is a supportive working environment with availability of (new) physical amenities and dental resources, and proper management and organizational structures with supportive visionary leadership. This would encourage teamwork and partnership development internally within the dental team and externally with its stakeholders. Furthermore, it would increase staff motivation and satisfaction internally and partnership development internally within the dental staff and externally with its stakeholders. Consider creating a new dental clinic. Develop a contract between the University and the Hospital to resolve the property ownership issues. Create a clear organizational structure and improve management interaction with clinical staff so that management is supportive. Improve the quality of dental supplies and create an efficient material ordering system. Ensure fairness to staff and respect to procedures are compliantly maintained to encourage teamwork. Reward clinical staff for mentoring and teaching dental students. Create access to continuing professional development for the clinical staff.

Key words: Access to care, allied health, utilization of health services, health management, oral health, dentistry, Papua New Guinea

INTRODUCTION:

Oral health is an important aspect of the general development and wellbeing of an individual [1,2]. To encourage and improve oral health, proper dental services with adequate resources are necessary. However, poor dental facilities and lack of resources is seriously affecting the quality of dental services being provided in both the rural and urban areas of Papua New Guinea (PNG). Of greatest concern is the dental service of the country's national referral and teaching hospital.

The Dental Clinic* is recognised as PNG's referral and teaching dental centre. Accordingly, it should include specialist services and be equipped with advanced health resources. The Dental Clinic has been sharing the clinic facility with the Dental School of a premier University*, under a special agreement since December 1989.

The unmet demand for dental services such as dental extractions and restorations is always a challenge to its providers and patients at the Dental Clinic. Yet, mid-2014 saw the closure of the main building that contained the ten out-dated dental units, due to medically-compromised issues. The Dental Clinic system faces a number of serious challenges, particularly service quality.

The definitions of quality medical services are diverse. Avedis Donabedian defines healthcare service quality as a process that maximises greatest benefit to humans without risk through the application of medical science and technology [3]. He distinguishes three components of quality: i) technical quality, ii) interpersonal quality, and iii) amenities [4]. Mosadeghrad's definition of quality healthcare service is "consistently delighting the patient by providing efficacious, effective and efficient healthcare services according to the latest clinical guidelines and standards, which meet the patients' needs and satisfies providers" [4]. Timeliness, consistency, amenities and facilities are some attributes of quality healthcare services, as identified by Mosadeghrad [5].

Dental services in PNG are far from efficient and, according to current global standard due to many contributing factors, including political and socio-economic issues [6-8], such as education level and income. Equity described as the freedom from favouritism, equality where people have equal access to services, and quality of dental services are ongoing major issues. Dental services in PNG are relatively scarce and expensive, and are utilized more in large urban centres than in rural areas [9]. A regional review of oral health in the Pacific in 1996 showed the total dental personnel to population ratio was 1:28,200 [10]. Current data

* To preserve the anonymity and privacy of participants drawn from a small professional group, details of participants and clinic are withheld

on dental personnel in PNG are unavailable to compare the variations, but inequity and inequality of dental personnel is still observed as a common problem in the Dental Clinic [9].

At present, there are no standards of care guidelines or policies developed for the dental profession in PNG [11-12]. While empirical studies have been carried out to assess the quality of healthcare organizations [13,14], little research has been conducted to identify factors that affect quality of healthcare services [4]. A limited number of studies have addressed this issue in both dental clinic settings [15,16] and in PNG [17]. The need for updated studies investigating the quality of dental services in PNG is essential to investigate this issue of quality services. This study, therefore, aimed to fill this research gap by empirically exploring dental providers' perspectives on factors affecting their dental service delivery in PNG organizations, in order to develop appropriate measures to increase the level of service quality [4].

The aims of this research were to identify and understand the factors affecting dental providers' delivery of quality service; and to explore possible measures that could aid in that outcome of quality dental services.

METHODOLOGY:

Purposive sampling was employed to identify the primary participants. The selected participants were those seen as having authority within the different cadres of the dentistry profession; currently working and clinically engaged with patients; that have access to the issue affecting the quality of dental services; speak English as their second language; and are from PNG. Those excluded were dental interns, because of their temporary placement within the organisation; and auxiliary staff, because they are not directly involved with the patient treatment process.

For a phenomenology research, 2 to 30 participants are considered sufficient to reach saturation or when additional interviews no longer reveal new information to the issue in this study [18,19]. Hence, a sample size of nine participants was selected initially. However, due to timing factors and patient workload during the interview week, a senior dental officer and an oro-maxillo-facial surgeon were not available for interviewing resulting in only 7 participants that were interviewed. The principal investigator contacted each of the identified potential participants directly through email, to discuss the nature of the research and seek permission for their involvement in the present research.

The Donabedian Model [20] and Haywood-Farmer's Attribute Service Quality Model [21] were used to govern this research project regarding dental service quality. Avedis Donabedian's [22] Model is widely accepted as the method for designing the main dimensions of healthcare quality. The model considers the viewpoint of structure, process, and outcomes. Haywood-Farmer's Attribute Service Quality

Model shares similar views to the Donabedian Model. However, his model states that a service organisation is of high quality if it consistently meets customer preferences and expectations [21]. His model describes services to have three basic attributes that form the apex of the triangle: (i) physical facilities and processes; (ii) people's behaviour; and (iii) professional judgement. Each attribute consists of several factors, and it is assumed that decline in service quality arises when there is too much focus on any one of these elements, leading to the exclusion of others [23]. Yerdavletova and Mukhambetov [24] suggested grouping these attributes in Haywood-Farmer's Model as direct and indirect factors that affect the quality of medical services.

The attributes in Haywood-Farmer's Model were grouped as direct and indirect factors that affect the quality of medical services [24]. These models are used to develop a theoretical model to investigate the key study variables in this research issue (Figure 1). Dentistry is a specialised medical profession that requires competent skills and theory, adequate resources, and advanced facilities to enable quality of services provided. Therefore, the proposed study variables consisted of dependent and independent factors of quality of dental services.

This research study used the naturalistic (critical) paradigm because no context or human experience being investigated is similar to the present research field of study. The methodological framework employed in this research project is the qualitative descriptive phenomenological approach. Semi-structured questions were developed. The questions were approved by the research supervisor and a pre-test was performed on two individuals with similar backgrounds to the participants.

An information sheet was provided to each participant prior to commencement of the data collection and consent forms were read and signed before any interviews were conducted. Ethics approval was obtained from The James Cook University Human Research Ethics Committee as well as endorsement from the Hospital's Management.

All interviews were digitally recorded in order to capture verbatim language and voice inflections; this process took approximately 45 minutes. The collected data and transcripts were saved on a secured password protected computer and backed up on a secure external hard drive. Transcripts were subjected to athematic content analysis [25]. Due to not having any analysis software, the whole processes of data analysis were hand coded.

Figure 1. Conceptual model: Factors affecting quality of service.

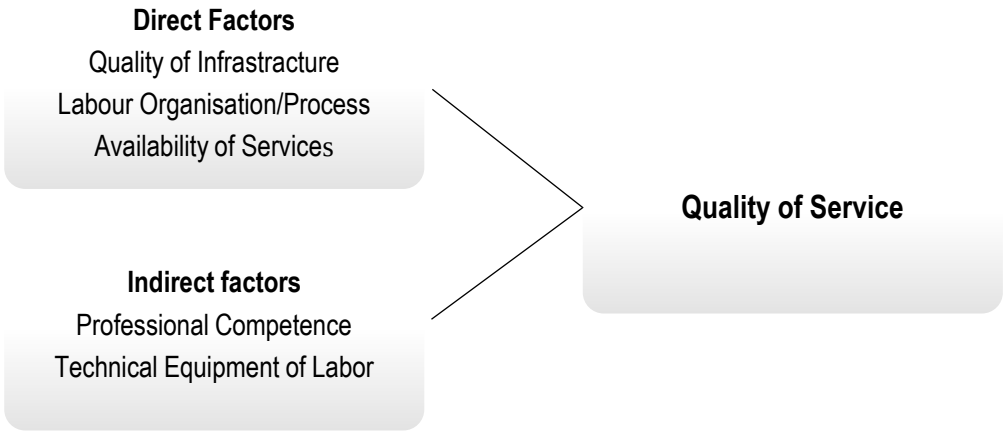

Table 1. Factors influencing the quality of dental service.

Category	Themes
Environmental Factors	Resources and Facilities
	Leadership and Management
	Partnership Development and Collaboration
Provider-Related Factors	Provider Competence
	Provider Motivation and Satisfaction
Required Measures	New Dental Clinic Building
	Renovation of Main Clinic Area
	Organizational Structure

RESULTS AND DISCUSSION

Due to timing factors and patient workloads, two were unavailable for interview leaving seven participants. Two senior dental nurses, one senior dental officer, one dental coordinator, one dental technician and two senior dental therapists were interviewed.

The views of participants on current factors influencing the quality of dental care services were grouped into three main categories and eight themes (Table 1).

Environmental Factors

Resources and Facilities: The non-availability and inaccessibility of dental materials and the issue of property ownership limited the providers' performances in service delivery, in terms of quantity and quality.

"You are in a university property, you are only allowed certain part of the building and so (inaudible)…yeah pretty much limited to what you have and what you can use so, but they've been very good in fact to allowing us to stay" (Participant 4).

"Yes, ah we have limited spaces, in the areas; at the room and um limited resources to better treat the patients" (Participant 6).

Leadership and management

Good leadership and top management support through strategic planning and the implementation of politically influenced polices were mentioned as an important enabler for progress of development of a new dental clinic building and/or renovation of the currently closed main dental clinic area. The lack of the Organization's visions for the Dental Clinic and its workers appeared to correlate to a strategic management disorder.

"But in the beginning they say there was funds for renovation but been diverted. So that's why I said we are not the priority people. So like priority has been given to other places so they divert the funding" (Participant 1).

"I'm sure the management is aware of it because our bosses, ah, they send report down but nothing has been happened" (Participant 3).

An organisation without proper structures to correct failures, either in its work force and/or environment, degrades staff morale, or causes interpersonal conflicts in the dental clinic. This was observed and commented by most participants as affecting their service quality in terms of motivation and interpersonal relationships.

"(Name withheld) comes to the clinic once a week or twice a week or sometimes not at all so in my opinion, the superiors should take note of these and give them some warning or letter of notice but there hasn't been any…" (Participant 2).

"Things that we need and we just complain to ourselves and we are not bringing our questions or queries to our supervisors because sometimes like we feel scared or we feel shy to tell them we should do this, we don't have this, we should be doing this" (Participant 3).

Conversely, most participants blamed the dental team's lack of collective or strategic approaches to ensure their voices were heard by the top management.

"You are only thinking about yourself and doing your own duties…. You all need to have a plan. Stop working aimlessly…" (Participant 2).

"I don't take part in decision-making of the place (long pause), management, they make the decision" (Participant 4).

Partnership development and collaboration

Low quality dental supplies and no proper structure of ordering and delivering were seen by participants as affecting their delivery of service quality in terms of tasks completions.

"*The Pharmaceutical services are the ones who are in charge of ordering. Before dentistry we choose our but when they make changes, it's solely the ah, procurement division blo (for) pharmaceutical service in health department. Yes, so they have been, I don't know whether they consult our office, curative health service up there, our department section up there or not" (Participant 5).*

"….we put our order through pathology and then we get (pause), sometimes like when we bring our container down, it takes time to come up. So sometimes like it's not really good that we ah, we have to use distil water for that autoclave machine" (Participant 3).

The Melanesian custom of wantok (one-talk) system and lack of task delegation were seen to be practiced in the Dental Clinic and this affected the dental employees' motivation to work. Effective communication and collaboration were considered essential parameters in high-quality healthcare service.

"So we are practising some kind of wantok (one-talk) systems in here, which is really not good. That's why things are not going on so well here" (Participant 2).

"There should need to shift, ah, it would require a total attitude change. A change of attitude from the staff; Ah now they are so relax because of the space wise, because there is no direction, everyone is relaxed. So they just wait for the people to, ah few operators to see the most patients while the others they just relax" (Participant 6).

Provider-Related Factors

Provider competence

Many participants commented that they were qualified and experienced to perform their job. However, the lack of resources and facilities that limits them to provide more advanced services.

"For myself, I feel that I am very experienced" (Participant 2).

"…we can do complex treatment in terms of, you know the restoration, of we've been doing that, yeah and we can do quality treatment just like a doctor" (Participant 5).

Participants were aware that patient care is their priority and, regardless of the working environment they had to ensure the patient's needs are met daily.

"Patient care comes first no matter the facility is not good or what, we still have patient care as our priority. That's what we're doing here" (Participant 1).

"Before you know when we got this paper (certificates) we said that through good or bad condition we will help (patients). We swore an oath" (Participant 2).

Therefore, to ensure providers provide the quality patient care necessary updates on the latest scientific and technological advances in this profession are necessary as mentioned by participants. As such, they saw the need for in-

services or workshops as necessary; however, they commented that this is no longer occurring.

"I think that's one of the main things also is lacking. They haven't been out of here to go for to attend some conferences and all that, you know workshops" (Participant 1).

"To have continuous education training using this facility, from this…to actually run an in-house based on what we actually need, and the technological and the scientific advances in material, that maybe we could advances" (Participant 4).

The interviewed participants commented about their willingness to establish a professional relationship with the University in terms of mentoring and teaching.

"The satisfaction to see that you are instilling sometimes the knowledge…the younger generations that can get out of you. Yeah so while being part of the hospital I think the most rewarding is actually teaching, yeah" (Participant 4).

"Like this dental, the (name withheld) should be practised on its own and like it's good that we are part of the university so that the students can learn from us…" (Participant 2).

However, participants have commented that they have stopped this practice of mentoring and teaching, due to lack of recognition or reward for their voluntary roles.

"….train the new and upcoming including you and the rest of the others first graduates. And that we did with heart, you know it was rewarding, you know getting it there where they were. Until there was, ah no, no understanding, no rewarding system, no saying thank you for…" (Participant 4).

Provider motivation and satisfaction

Dental providers identified good working environment, good leadership, and transparent organisation visions as factors that influence the dental providers' motivation and, consequently, job satisfaction.

"And you can see from the space where we working at the moment, we only have three chairs and we are the referral hospital and the national referral hospital. And we are not even doing that. We seem to be doing the simple (treatment), so the services are not good" (Participant 1).

"The most exciting part of my work is like I enjoy working with the doctors especially when we are doing surgical cases" (Participant 7).

Almost all participants expected at least some form of organization structure to guide staffs' performance in work. They commented that having a goal would increase staff motivation in their service delivery.

"But if they can give us some goals to achieve, then I believe we should provide as many services in our, ah there are other services like um, education, oral education to help ah to prevent, which is not taken place" (Participant 6).

"Since they remove the chairs on the other side, ah no, there is no real development even though the hospital promised us. Ah well it is, I

mean its hospital's responsibility to do the maintenance, ah infrastructure, that's all is hospital's responsibility" (Participant 5).

"Plus like now the training, they've stopped helping out the training for our staff from (name withheld). So if you want to do training, you have to resign" (Participant 1).

Required Measures to Improve Service

Currently the main measures required to improve the services provided, as voiced by the participants are the need: for a new dental clinic building fully equipped with necessary dental equipment and resources; to renovate the main clinic; and of a good management.

New Dental Clinic

All the participants were certain that a new dental clinic, equipped with appropriate technical dental materials, would boost staff morale to work and raise the quality of service provided.

"Yes like I do enjoy working in the surgery but most of the time we don't have you know proper instruments and we should have our own dental (pause), my point of view like we should have our own dental clinic. We should have everything, dental lab, wards" (Participant 7).

"Yeah basically the facilities and having a clinic where you can actually relax and see your patients daily as they come instead of sharing and you know, there's a (incomplete sentence)" (Participant 4).

Renovate main clinic

There were concerns with the long process in decision-making in approval for a new dental clinic. It was suggested that renovation of the main dental clinic building and instalment of new dental chairs would be less costly, require less decision-makers and should be the current priority.

"And they have to approve and that will take a very long time. So because the building is here, all we want is to see, ah implemented quickly is just get the chairs and refurnished the place again and let's see the patients…" (Participant 5).

"And of course the chairs are all run-down so we need new chairs. And that is one of the things we're really trying to work together with the management to help us to continue the renovation" (Participant 1).

Organisational Structures

The participants commented that a clear organisational structure involving task delegation, update of knowledge and skills, clear visions and goals were necessary to improve the service delivery.

"Management to lead the proper management to our setting to a dental level, and also to a national level. That is actually what's missing and collective approach to get everyone involves" (Participant 4).

"We just come see patients and then we go so there should be something put in place like a strategy plan like in the next 5 years we should achieve somethings." (Participant 6).

Availability of dental resources, including facilities, equipment, materials and staff, which includes clinical and non-clinical workers [26], was seen by participants to play an important role in delivery of a quality dental service. This finding is similar to that found in previous research [4,15,27]. These findings are of great importance to organizational managers as they identify factors that influence employee commitment and motivation.

The implementation of the Free Health Services in PNG and the lack of the much needed government support during the implementation of this policy have affected the financial budget of the Hospital. The Hospital's budget is insufficient to purchase the many instruments and materials that are needed to ensure quality patient care outcomes and services from the dental providers. This has resulted in limited treatment options being provided to a limited number of patients. The only treatment currently offered was relieving of pain via teeth extractions.

Although an improved working environment is necessary [28], other factors, including social support and encouragement for innovation at work, are equally important for enhancing providers' feelings of dedication and motivation to work [4,29,30]. These can be easily achieved through the development of an effective organizational structure within the dental setting.

Types of leadership and management differ in different settings; however, effective management will enable the progress of development and encourage teamwork within any organisation [4]. These suggest that dental service quality can be improved by a supportive or quality-orientated management, with clear goals and objectives, and strategic planning and management of resources, including employees and processes. These are consistent with other findings of creating an inspiring vision, establishing shared values, setting clear goals and objectives, providing direction, motivating employees and empowering them to participate actively in quality improvement, monitoring progress, providing appropriate feedback and giving tangible rewards [4,31].

Supporting services and resources such as suppliers, logistics or cleaning services are crucial in the effective and efficient delivery of any business or organization's operations. In dental services, as with any other healthcare service, maintaining good professional and encouraging collaboration with other healthcare providers and/or support businesses is necessary. For instance, it is essential that the Dental Clinic establishes a good feedback relationship with its dental suppliers to ensure that the correct and accurate instruments or equipment are ordered and purchased. Working in collaboration with the Pharmaceutical Services is necessary to ensure quality dental materials are supplied, or providing case reports on the issues of lack of water supply in the sterilising room in order to

develop an efficient strategy plan in the delivery of distilled water.

The need for constantly updating knowledge and skills is vital to provide quality, reliable and accurate treatment options for patients, due to the rapid development of advanced technology and scientific findings in medical professions [4,32,33]. However, the issue that is currently impeding dental workshops and in-house trainings in the Dental Clinic is the lack of finance. A continuous organisational learning team should be organized within the Dental Clinic team to meet further employees' professional and skills needs.

Another issue raised during the research included the training provided to the dental students. Training takes time, expertise, and finance, therefore, institutions providing such should play a bigger role in the effective training of human resources for healthcare organisations. Staff liked mentoring and teaching students. However, the need for recognition or acknowledgment was important to influence this attitude. This finding was consistent with other research that appropriate recognition and reward systems are important tools for employees who want to improve the quality of their work [32,34].

Recognition and the feeling of achievement are more likely to influence staff motivation and, thus, their performance [35] as commented by some participation during the interviews. Findings found that participants could be very competent, but without much or due recognition could affect service quality through poor work performance or low work inter-personal relationships [33]. Resource availability and worker competence are essential, but not sufficient to ensure desired worker performance [35,36]. Despite the poor working environment, the factor of few dental employment opportunities in the country has prevented most staff from leaving the Dental Clinic in search of better job opportunities. Hence, the more appropriate approach would be to create a united workforce to ensure staffs are committed to providing the highest-quality care.

Several limitations need to be acknowledged. The results should not be generalized to other countries or health organizations with different organizational characteristics, business culture and management styles. Secondly, due to the deadline of this research project task it was impossible to conduct different data collection methods and to develop and evaluate action for change. Not all participants were available for the interviews, and the non-available people may have had different views that could have affected the result findings [37].

Further research is encouraged to use this method in conjunction with quantitative methods. It is recommended that future studies in this setting may want to explore and identify factors following the development and implementation of a quality management plan, by using a mixed-method approach. Ultimately the client should have a say in determining

service quality and this is also a necessary area for further research.

CONCLUSION AND RECOMMENDATIONS:

The factors influencing the quality of dental service fell into three categories and 8 themes.

Lack of quality management and strategic planning affected the progress of dental service delivery, and had an indirect effect on staff members' morale and motivation to work. Staff members have highlighted that these issues affected their services many times. Therefore, effective leadership and team approaches will support employees by encouraging education, self-development and removing barriers between staff and their superiors.

The recommendations includes consider creating a new dental clinic that is equipped with technical dental materials; develop a contract between the University and the Hospital to resolve the property ownership issues; create a clear organizational structure and improve management interaction with clinical staff so that the management is supportive; improve the quality of dental supplies and create an efficient material ordering system; ensure fairness to staff and respect to procedures are compliantly maintained to encourage teamwork.; reward clinical staff for mentoring and teaching dental students; and create access to continuing professional development for the clinical staff.

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CASE REPORT:

ISOLATED PROGRESSIVE CONGENITAL LEFT THUMB MACRODACTYLY: CASE REPORT AND LITERATURE REVIEW

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ABSTRACT:

Isolated progressive macrodactyly belongs to a heterogeneous group of congenital overgrowth syndromes, resulting in enlargement of all tissues localized to the terminal portions (fingers or toes) of a limb. The aim of this case report is to create further awareness among physicians on this rare clinical entity and present a short review of the literature. We present the case of a 10-year-old Nigerian girl with a history of progressive overgrowth of left thumb since birth. The patient's facies and body habitus were normal without hemihypertrophy. Anthropometric measurements of the child's hands, revealed an overgrowth of the left thumb compared with the right. The child had no café-au-lait spots or any other skin lesions. The radiograph of the hands revealed increase in size of both soft tissue and phalangeal bones (enlongated and broadened) of the left thumb. A diagnosis of isolated progressive left thumb macrodactyly was made and the patient was referred to the orthopaedic surgeon for surgical intervention. The child and her parents suffered psychological distress. Isolated progressive macrodactyly is very rare but it is cosmetically displeasing to the child, resulting in psychological distress.

Key words: Congenital, localized gigantism, macrodactyly, overgrowth syndrome, Nigeria.

INTRODUCTION:

Macrodactyly is a rare group of congenital anomaly characterized by the enlargement of one or more of the fingers or toes in the absence of other lesions. The overgrowth involves all the digital structures (nail, tendons, intrinsic tissue, joints and phalanges) in the true variety [1]. False macrodactyly presents as hypertrophy of primarily one tissue type [2]. The incidence in one active paediatric orthopaedic clinic in USA was 0.035%, with a slight male preponderance (male: female ratio

1.2:1) [3]. Macrodactyly represents 0.9% of all congenital anomalies of the upper limb [4]. There is no familial inheritance pattern and it usually presents as an isolated, non-syndromic condition [5].

According to the classification proposed by Upton [6], there are four types of macrodactyly: Type I is macrodactyly with lipofibromatosis of nerve, either static or progressive subtype; Type II is macrodactyly associated with neurofibromatosis; Type III has associated hyperostosis; and Type IV is associated with hemihypertrophy. A true macrodactyly may also be associated with other syndromes such as Proteus, Beckwith-Wiedermann and Klippel-Trenaunay-Weber syndromes [1,7]. In the static type the enlarged digit is present at birth but its growth is proportional to the growth of the child's other fingers whereas in the progressive type, it may or may not be present at birth but the growth of the involved digit is in excess compared to the growth of the child's other fingers [3]. Isolated macrodactyly belongs to a heterogeneous group of congenital overgrowth syndromes, resulting in enlargement of all tissues localized to the terminal portions of a limb, typically within a 'nerve territory' (nerve-territory-oriented macrodactyly) [8,9]. The classic terminology for this clinical entity is lipofibromatous harmatoma of nerve or type I macrodactyly [9]. The affected peripheral nerve itself is enlarged both in circumference and length. In the hand, the reported clinical distribution of macrodactyly was as follows: thumb 9%, index finger 20%, middle finger 34%, ring finger 22% and small finger 15% with slight male preponderance 1.0:0.66 [10]. In that study, there was no laterality or gender difference and only 5 of 33 cases analyzed involved isolated digits [10].

The pathogenesis of isolated macrodactyly has not been fully elucidated. It has been associated with lipofibromatosis of peripheral nerve, most commonly the median nerve [11-14]. In Lebanon, Fares et al [11] reported a case of an 18-year-old boy with neural fibrolipoma of the median nerve causing progressive macrodactyly of the left thumb. Recently, isolated macrodactyly has been linked to activating somatic mutation in the phosphatidylinositol-3-kinase catalytic alpha (PIK3CA) gene and a new term referred to as "PIK3CA-Related" Overgrowth Spectrum (PROS) has been proposed to accommodate the broad range of clinical manifestations in these patients [15]. Using whole-exome sequencing, Rois et al [9] identified somatic mutations present in the affected nerve of a single patient. In that study, they confirmed a novel mutation in PIK3CA in the patient's affected nerve tissue. In the same study, immunocytochemistry confirmed AKT activation in cultured cells from the nerve of a patient with macrodactyly. They stated that isolated congenital macrodactyly is caused by somatic activation of the PI3K/AKT cell-signaling pathway and is genetically and biochemically related to other overgrowth syndromes [9]. In

another study in USA, it was found that a developmental cytokine, pleiotropin (PTN) was significantly overexpressed across all samples obtained from patients with macrodactyly [5]. The authors stated that the function of PTN correlated closely with the clinical characteristics of macrodactyly [5].

Some clinical conditions have been associated with macrodactyly. Kalen et al [3] reviewed 167 cases and reported one case with neurofibromatosis, one with adrenal tumour and hemihypertrophy, one with epidermal naevus, one with multiple connective tissue nevi, and one with epiphysealis hemimelica. In addition, macrodactyly involving both hands and one foot associated with cutaneous haemangiomata has been reported [7]. In Brazil, a 15-year-old boy with macrodactyly co-existing with skin hypertrophy was reported by de Almeida Jr et al [16].

Let us consider the natural history of macrodactyly. With advancing age, the enlarged curved phalanges lead to osteophyte formation which causes early joint degenerative disease and pain [7]. In the affected digit, bony growth continues until epiphyseal closure but soft tissue enlargement may continue into adulthood [17]. With time, the digit enlarges and motion decreases, resulting in functional impairment [3]. According to Kalen et al [3], the basis of the functional impairment is that the soft tissue hypertrophy on the palmar surface of affected digit causes the distal interphalangeal joints to be hyperextended, preventing flexion of the digits, resulting in impairment of pincer movement where the thumb is the finger affected.

The purpose of this case report is to create further awareness among physicians on this rare clinical entity and present a short review of the literature.

CASE REPORT:

A 10-year-old Nigerian girl was referred to the Endocrinology and Metabolism Clinic of the University of Benin Teaching Hospital (UBTH) with a history of enlarged left thumb since birth. The left thumb progressively became larger (out of proportion to the right thumb) as she got older. There is no associated pain. Family history was negative. The school mates and neighbours ridiculed her and this prompted parents to seek for medical attention for their child. The patient had no other medical problem.

The patient's facies and body habitus were normal. Her anthropometry showed: weight 25.5kg (10th percentile), height 137cm (50th percentile), arm span 143cm , upper segment:lower segment ratio 0.9 and body mass index 13.6kg/m2 (3rd percentile). The left thumb was enlarged but non-pulsatile. The measurements of the fingers were as follows: The left thumb measured 9cm in length (measured from proximal crease to tip of finger) and 8.7cm in circumference. The right thumb measured 4.5cm in length and 5.5cm in

circumference. Further examination of the hands revealed macrodactyly of left thumb with hyperextension and abduction (Figure 1). The left thumb nail was enlarged compared to the right (Figure 1).

The other fingers and toes were not affected. Neither syndactyly nor polydactyly was present. The child had no café-au-lait spots or any other skin lesions. The remainder of the physical examinations was unremarkable. The radiograph of the hands revealed increase in size of both soft tissue and phalangeal bones (enlongated and broadened) of the left thumb (Figure 2).

Abdomen/pelvic ultrasound did not reveal any abnormality. A diagnosis of isolated progressive congenital left thumb macrodactyly was made (corresponding to type I macrodactyly). Subsequently, the patient was referred to the orthopaedic surgeon for surgical management.

Fig. 1: A photograph of the hands of a ten-year-old girl showing macrodactyly of left thumb

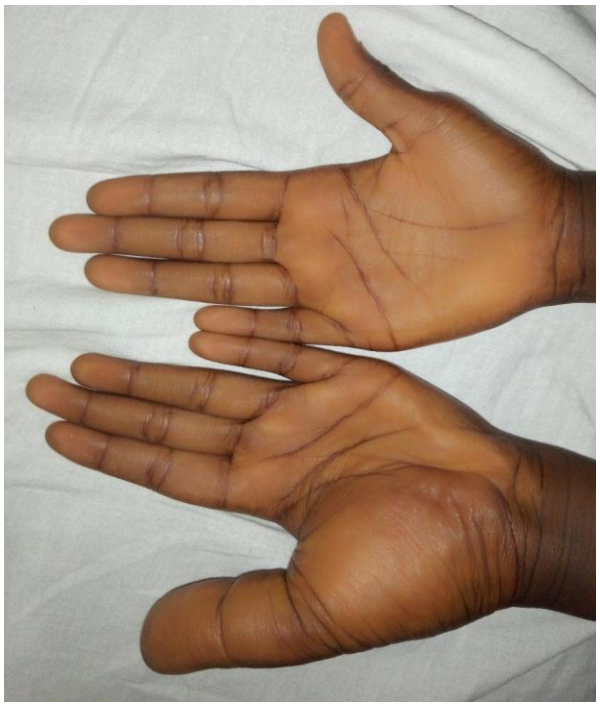
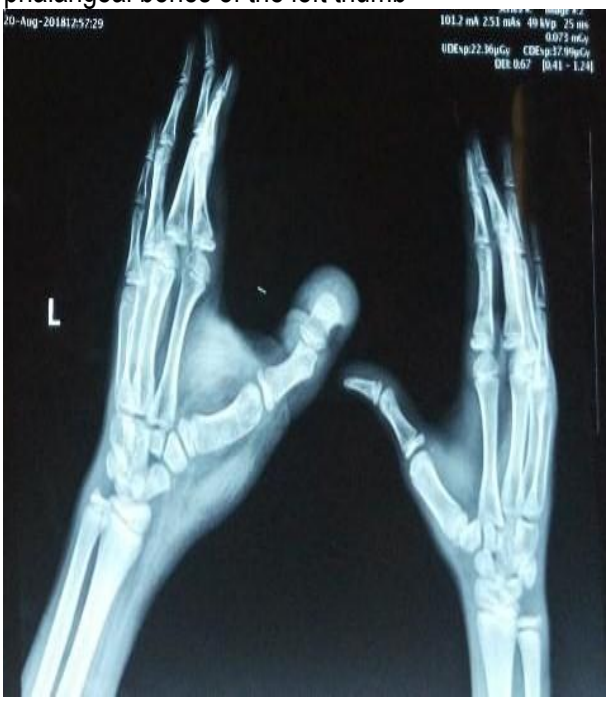


Fig. 2: A radiograph of the hands of a ten-year-old girl showing increase in size of both soft tissue and phalangeal bones of the left thumb



DISCUSSION:

As in our patient, localized gigantism is often recognized at birth but begins to cause problem as the child grows. In the index case, the diagnosis of isolated progressive congenital left thumb macrodactyly was made based on

history, physical examination and radiologic findings. This is in consonance with method of diagnosis employed in other studies [18,19]. Increase in size of the phalanges has been noted as a primary feature of the anomaly with or without the involvement of the metacarpals [20]. Our patient had evidence of overgrowth in the left thumb when compared to the right thumb. The increase in size of the affected digit involved both the soft tissues and the phalangeal bones as demonstrated in our patient's left hand radiograph. Although macrodactyly is a rare clinical entity, its diagnosis is straightforward. The rare nature of the condition is evident from the report of a retrospective review involving an active paediatric orthopaedic clinic in USA where only 21 cases were seen over a period of 15 years [21]. In United Kingdom, 41 cases were seen over a period of 17 years [10]. The report of a study conducted at one of Nigeria's National Orthopaedic Hospitals revealed that of the 43 patients with anomalies of the hand only one had macrodactyly of the middle finger [22], further supporting its rarity. Indeed, it has been reported that macrodactyly is rarer in Blacks than Caucasians but the author did not provide any explanation for this racial difference [23].

Some of the clinical features in our patient are in keeping with those reported in literature in patients with macrodactyly. The index case had soft tissue hypertrophy on the palmar surface of affected digit as well as over the thenar eminence, hyperextension and abduction of the affected left thumb. The thumb nail is also enlarged. The patient did not have difficulty with performing pincer movement (able to pick up a pen from the table, using thumb and index finger), suggesting that functional impairment of the affected thumb is yet to set in. The apparent preservation of function may be explained by the report of Yang et al [24]. In that study, the authors concluded that although the morphology and function of the median nerve was impaired, the unaffected ulnar nerve partially compensated for the lost function of the affected median nerve under certain conditions. The results of some studies indicate that in macrodactyly, the index finger is the most frequently affected of all the fingers [3,23] but it was the thumb that was affected in our patient, supporting the recognized heterogeneity of clinical phenotypes [9]. Our patient presented at the age 10 years but in one Nigerian study, the reported peak age at presentation was 0-11 months [22].

The negative family history in our patient is in keeping with the sporadic nature of isolated macrodactyly. Both the patient and her parents suffered psychological distress because of ridicule by school mates and neighbours. This prompted parents to seek for medical attention for their child. This scenario is not surprising because concealing macrodactyly of the finger is difficult.

The anatomic extent of involvement varies from one or more digits to entire hand or foot. In addition, the rate of growth differs from

patient to patient [5,9]. According to the report of Rois et al [9], the observed phenotypic variability of PI3K-AKT-associated overgrowth syndromes might be due to the timing of the mutational event during development as well as tissue specificity, leading to distinctly different disease syndromes.

Although isolated progressive congenital macrodactyly is a benign condition, it is deforming and cosmetically displeasing to the child, siblings and parents. The reports of other studies have cited displeasing cosmetic appearance as the main reason for seeking medical attention [19]. Given that macrodactyly exhibits varying clinical phenotypes, there is no defined treatment algorithm. Therefore, treatment is usually decided on individualized basis. According to Carty et al [25], the treatment principles in the index case are those of provision of an esthetically acceptable hand with pincer capacity for gripping objects. In the same report, the authors stated that because of the uncertainties surrounding the outcome of surgical intervention, the International Federation of Societies for Surgery of the Hand described macrodactyly as an 'unsolved condition'. In a retrospective study involving 32 patients in Birmingham Children's Hospital, Hardwicke et al [10], concluded that in vast majority of cases the functional and cosmetic outcomes were good, with good patient acceptance. The paediatric patient with macrodactyly may require additional surgical procedures because the deformity will continue to grow. However, early treatment will provide for the affected child the benefit of functional and cosmetically appealing hand.

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LETTER TO THE EDITOR:

INFECTIOUS ENDOCARDITIS ASSOCIATED TO PERMANENT PACEMAKER: APPROACH FOR LEAD EXTRACTION WITH LARGE VEGETATION.

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Short Title: pacemaker lead extraction with large vegetation.

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Dear Editors,

A 70-year-old female consulted for a high fever of 6 days duration that exhibited no focality. The only pathological history referred unicameral pacemaker implantation 6 years ago (unknown cause) and arterial hypertension. Our patient had no history of others disease. On admission she had a temperature of 38.5°C. She did not show any relevant data at physical examination. Laboratory results showed leukocytosis and increased erythrosedimentation. Hemocultures were positive for methycyline-sensible Staphylococcus aureus. Upon suspicion of infectious endocarditis (IE), a transesophageal echocardiogram was requested; where at the atrial level on the catheter was observed with a vegetation mobile image (Fig. 1A). After confirmed diagnosis of catheter-associated IE, the recommended therapy was implemented. Complete removal of the device was performed percutaneously (no pacemaker-dependent patient). For percutaneous removal of the system, locking stylets (Cook, Liberator) and polypropylene telescopic sheaths were used (Fig. 2). Eight hours later she presented dyspnea and tachypnea with normal oxygen saturation and stable blood pressure.

Computed tomography scan of the chest demonstrated images compatible with pulmonary infarction (Fig 1B) associated to isolated subsegmental pulmonary embolus.

She completed the course of antibiotic (cefalotine for 6 weeks) and the new device was reimplanted. The patient not presented cardiovascular or infectology complications after two-year follow-up, IE associated with permanent an implantable electronic device is an uncommon complication, but with high mortality levels if inadequately treated [1]. It

involves long-acting intravenous antibiotics and complete removal of the device either percutaneously (dilator sheaths, locking stylets, etc.) or, if this is not feasible, surgical removal is performed by thoracotomy and extracorporeal circulation but presented high mortality rate. Progress in techniques and materials for intravenous removal of catheters by experienced operators are the basis of the low rate of procedural complications.

The vegetation size is a controversial issue, as some authors believe that transvenous or percutaneous removal of catheters with vegetations of up to 20 mm poses the risk of pulmonary embolism [1, 2, 3]. Other publications have shown that tranvenous removal is safe in patients with infective endocarditis associated with permanent implantable electronic devices and vegetations of up to 20-mm [4]. At present, there are still discussions regarding patients with infective endocarditis associated with permanent implantable electronic devices and vegetations exceeding 20 mm.

We know that there is a high rate of pulmonary embolism (33%) transvenous removal in IE associated with permanent an implantable electronic device, it is asymptomatic or mildly symptomatic in most cases [5]. Several symptomatic cases (embolism with higher vascular involvement) are less common. The mortality directly related to the CIED extraction procedure ranged from 0.4% to 3.6% [5].

The decision is influenced not only by the vegetation characteristics, but also by the patient's prior cardiopulmonary condition, another concomitant heart surgical prescription, (atrial communication with the risk of paradoxical embolism and the possibility of complete removal of the device will be evaluated.

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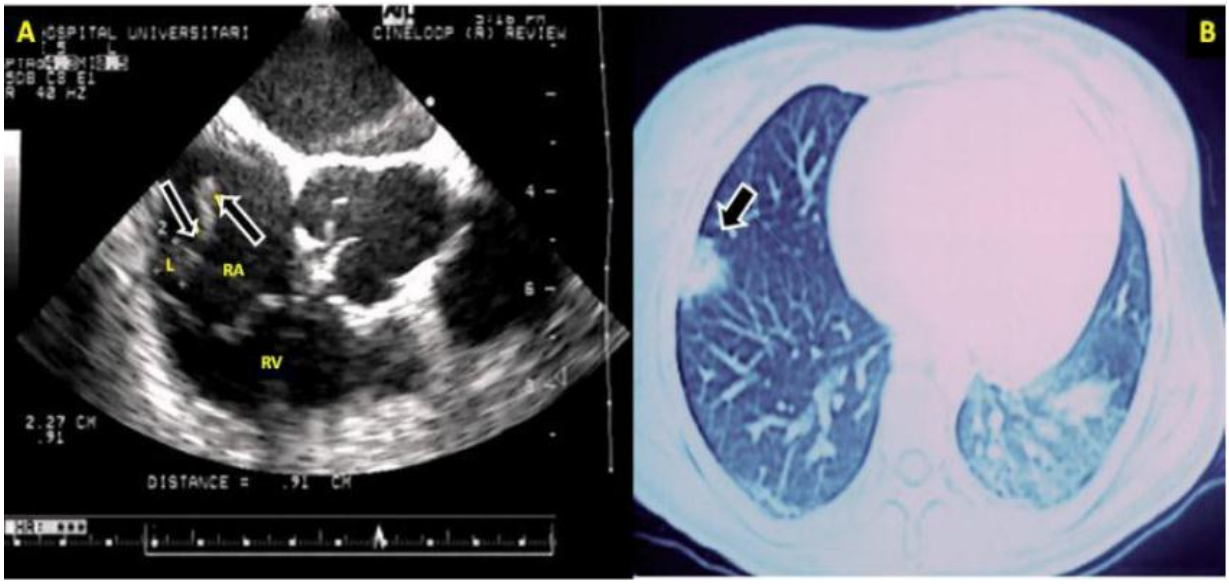

Figure 1: A) Large vegetation seen with transesophageal echocardiography at the atrial level on the catheter. The largest longitudinal diameter is 22 mm (arrow). RA: right atrial; L: lead. VR: ventricular right. **B)** Axial views of a computed tomographic on lung window (Post extraction). The infarct in this computed tomographic scan is represented by the wedge shaped density in the right lung with its base abutting the pleura (arrow).

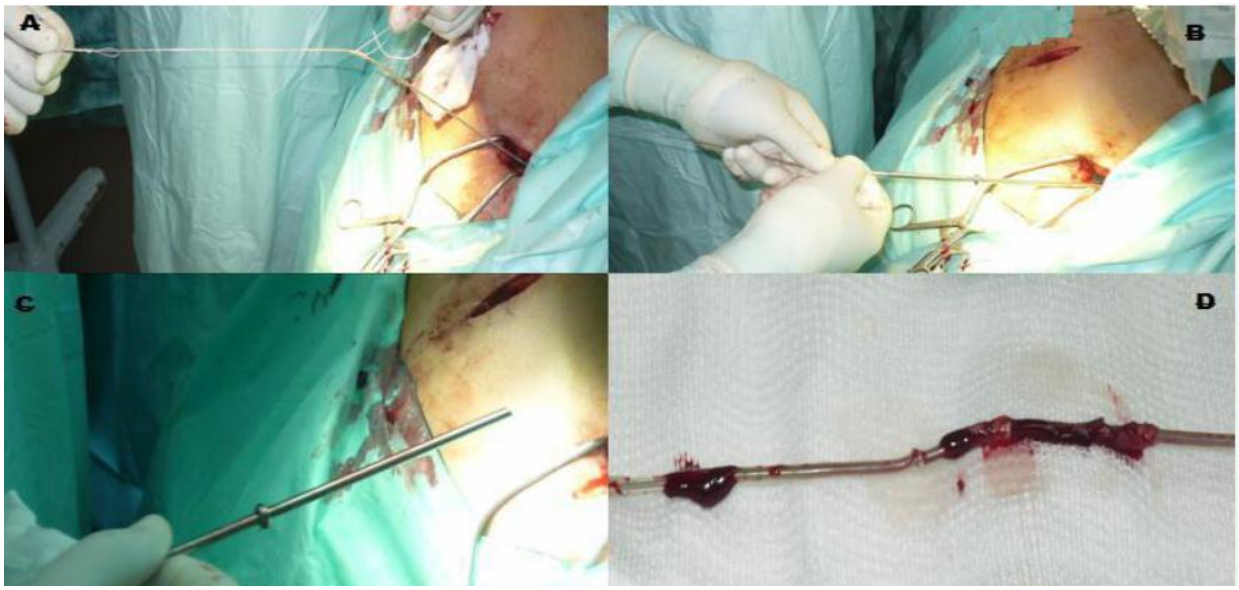

Figure 2: Extraction procedure: (A) Placement of the locking stylets (Cook, Liberator). B) Insertion of the rigid. C) Withdrawal of the sheath and the catheter simultaneously. D) Ventricular catheter removed in the proceeding with vegetation attached.

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