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The Tropical Journal of Health Sciences (TJHS) is an international journal which provides a forum for exchange of ideas among those engaged in the Health Sciences and related fields. The journal intends to publish high quality papers on original research, case reports, short communications, commentary, review articles, editorials, correspondence and book reviews. TJHS is an official organ of the College of Health Sciences (A WHO Collaborating Centre for Research and Manpower Development), University of Ilorin, Ilorin, Nigeria and will also serve as a medium for disseminating information on the activities of the College.

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THE TROPICAL JOURNAL OF HEALTH SCIENCES

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EDITORIAL COMMENTS

This edition covers disciplines in basic medical research and clincal practice. The first article focused on the mechanochemical synthesis and characterization of trimethoprim - copper complex and its antimalarial efficacy on *Plasmodium berghei* infected mice. Results indicated that coordination of copper to trimethoprim by mechanical induction improved its antimalarial activity but may not be completely safe due to alterations in the investigated biochemical parameters.

The second article discussed histomophological evaluations and wound healing efficacy of honey in comparison with *Vernonia amygdalina* Leaf Juice. Similar to honey, *Vernonia amygdalina*leaf juice, enhanced fibroblasts recruitment, epithelia cells migration, neovascularization and reduced polymorphonuclear leukocytes (PMNL) infiltration as compared to the control animals at the early phase of wound healing.

The third article and the first case report in this edition focused on vision, the third article determined the prevalence of abnormal Photostress recovery time (PSRT) and the association of PSRT with age, visual function and driving safety among commercial drivers in Nigeria. The study revealed a statistically significant association between PSRT and visual acuity as well as color vision and no association existed between PSRT and age, visual field and driving safety. The first case report described presentation, diagnosis and management of Herpes Simplex Virus Keratitis infection in a 25 year old Nigerian male.

The fourth and fifth articles focused on pregnancy. An article from Ibadan discussed the concentrations of micronutrients and heavy metals in cord and maternal blood of pregnant women using electric stove compared with firewood users. The results revealed no significant differences in the levels of micronutrients and heavy metals in cord sera of babies born by mothers using electric stoves compared with firewood users, but Zn level was significantly higher whereas Pb level was significantly lower in sera of mothers using electric stoves compared with mothers using firewood. It's concluded that low Zn and high Pb might be due to adverse effects of firewood on the users. Following this trend, another article discussed the knowledge and attitudes of pregnant women towards anaesthetic techniques for caesarean delivery in a Nigerian teaching hospital. Results revealed high awareness level about anaesthetic techniques for caesarean delivery among the parturient. The preferences of parturient for specific anaesthetic techniques favoured regional anaesthesia (spinal) compared to general anaesthesia.

The sixth article described the consequence of misuse of oral hypoglycaemic agent in patients with lower urinary tract symptoms in Ilorin, Nigeria. Case series of five patients with lower urinary tract symptoms, who had abused oral hypoglycaemic agents resulted in various stages of altered consciousness and frank hypoglycaemic coma. An article discussed the need for adequate regulation on the anatomical practices of preserving, handling, managing and disposing of human remains and a proposition for an act in the laws of Nigeria.Stating that changes and advancements in societal life and circumstances have limited the practicability of some aspects of the old Anatomy Act, especially as it relates to the management of human remains for non-dissection educational purposes.

Last is a case report discussing the management of Dandy-Walker Syndrome in an adult patient. Dandy-Walker Syndrome (DWS) is acongenital brain malformation involving the cerebellum and the fluid spaces around it. Symptoms such as delayed developmental milestone, bulging of skull may present early in life or may present later in life. The patient was managed successfully with cysto-periotoneal shunt insertion under general anaesthesia without postoperative ventilation.

AB Okesina
Editor in Chief

Safety Evaluation and Antimalarial Effect of Mechanochemically Synthesized Trimethoprim-copper In *Plasmodium berghei* Infected Mice

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Abstract

Antimalarials are central to any strategy for effective reduction of malaria-related mortality. Efficacy and safety of antimalarial medicines, as measured by their quality, are therefore essential in mitigating morbidity and reducing deaths. The mechanochemical synthesis and characterization of trimethoprim-copper complex and its antimalarial efficacy on *Plasmodium berghei* infected mice and toxicity evaluation were investigated by evaluating percentage parasitemia and chemosuppresive effect of the drugs on *Plasmodium berghei* infected mice, status of some serum enzymes and hematological indices. Derivatization of trimethoprim with copper enhanced the activity of the drug by significantly ($p<0.05$) improving the suppression of parasitemia in established infection when compared with the controls. Trimethoprim-copper complex demonstrated to be more efficacious than pure trimethoprim while chloroquine was most efficacious in malaria parasite clearance. Administration of trimethoprim, trimethoprim-copper complex and chloroquine to mice for seven days caused significant increase ($p<0.05$) in the activities of alkaline and acid phosphatases in the liver, kidney and small intestine when compared with the control while a significant reduction ($p<0.05$) in the activities of alanine and aspartate aminotransferases, and lactate dehyrogenase were observed in the liver, kidney and small intestine when compared with the control. Also there was a significant decrease ($p<0.05$) in the levels of PCV, Hb, RBC and lymphocytes and a significant increase ($p<0.05$) in WBC and neutrophil concentrations in all the treatment groups when compared with control. The result indicates that mechanical synthesis of trimethoprim-Cu improved trimethoprim activity while the alterations in the investigated biochemical parameters suggest functional and structural toxicity.

Key words: Mechanochemical synthesis, antimalarial activity, mice, trimethoprim, safety.

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Introduction

Malaria is one of the most prevalent parasitic diseases in the world. It is transmitted to humans by the female Anopheles mosquito and affects approximately 500 million individuals throughout the tropical and subtropical areas of developing countries and causes considerable morbidity and mortality with about 800,000 deaths worldwide each year [1]. Malaria is caused by different species of *Plasmodium*, *Plasmodium vivax*, *Plasmodium falciparum, Plasmodium malariae* and *Plasmodium ovale* of which *P. falciparum* is the most virulent human malaria parasite [2] (Sehkon and Bimal, 2012). The drug of choice for the treatment of all forms of malaria in many societies is chloroquine (CQ). However, CQ is no longer used alone due to the emergence and spread of *P. falciparum* CQ-resistant strains and, more recently, of P. *vivax* [3,4,5]. Artemisinin based combination therapy was the first line of treatment in *P. falciparum* malaria (WHO, 2009). The decreased susceptibility of *P. falciparum* to artemisinin derivatives [6,7] have required the search and development of novel anti-malarial drugs [8,9,10] and a careful re-examination of the existing drugs [11,12,13].

One of the main goals of present day organic coordination chemists and pharmaceutical investigations is the discovery and development of better and cheaper drugs to fight diseases, and this has led to numerous studies on drug metal complexes [14,15].

Various studies have been carried out on complexation of some common antimalarial drugs with metals [16]. The efficacy of the therapeutic agent is known to be enhanced upon coordination to a metal ion. The ability of metals to combine with ligands and then release ligands in some processes make them ideal for use in biological systems. Many drugs possess modified toxicological and pharmacological properties when in the form of metal complexes [17]. It is not surprising that antiplasmodial efficacy proved to be somewhat dependent on both metals and ligands [18].

Substituted 2, 4 diaminopyrimidines are widely employed as metabolic inhibitors of pathways leading to the synthesis of proteins and nucleic acids. Among these kind of ligands, trimethoprim (trim 2, 4-diamino-5-(39, 49, 59-trimethoxybenzyl) pyrimidine) is a well-known biological agent employed as a potent metabolic inhibitor of bacterial dihydrofolic acid reductase. This drug is an antibacterial agent highly active against strains resistant to other antibiotics

frequently used, e.g. β-lactam antibiotics. Dihydrofolate reductase, DHFR, whose role is to regenerate folic acid into its reduced form-tetrahydrofolate, is necessary for bacteria, plasmodia, normal and cancerous human cells. Inhibitors of dihydrofolate reductase have antibiotic, antimalarial and antineoplastic properties. Several authors have studied the interaction of this ligand with biological metal ions and the coordination of trimethoprim via a NH nitrogen atom was inferred on the basis of IR and visible measurements [19]. However, other authors have shown by X-ray diffraction methods that the coordination site of the trimethoprim molecule is the N atoms 5 and 9 of the pyrimidine ring. On the other hand, Seekhon *et al.*[20] have prepared and characterized complexes of trimethoprim with Ag(I), Zn(II), Cd(II), Hg(II) and Ni(II) and the infrared data revealed that the ligand acts as a monodentate through the 4-NH group.
In this study, we investigated the solvent-free synthesis of a trimethoprim-Cu complex using a mechanochemical method and its *in vivo* antimalarial activity and safety.

Materials and methods

Source of reagents

The albumin, total protein and creatinine, AST, LDH and ALT assay kits were products of Randox Laboratory Ltd, Co-Atrim, UK while that for chloride ion is a product of Quimica Clinica Applicada Ltd, Amposta, Spain. The assay kits for sodium and potassium ions are products of Agappe diagnostics Ltd, Kerala, India. All other chemicals and solvents used were of analytical grades and were used without further purification. They were obtained from BDH Chemicals, England.

Source of experimental animals and parasites

Thirty healthy albino mice (*Mus musculus*) (24.90 ± 2.00g) with age range of 8-11 weeks were obtained from the animal breeding units of the Department of Biochemistry, University of Ibadan, Oyo state, Nigeria. The animals were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad-libitum*. The studies adhere to the Principles of Laboratory Animal Care (NIH, publication number 85 – 23, revised in 1985). The parasite used was a chloroquine-sensitive strain of *P. berghei* NK 65 [21], maintained in mice, obtained from the Faculty of Clinical Pharmacy, Obafemi Awolowo University, Ife, Nigeria.

Synthesis of Trimethoprim Copper Complex

Copper acetate ($Cu(CH_3COO)_2.H_2O$) and Trimethoprim (0.880g, 2.0 mol) were carefully weighed into Retsch MM200 stainless steel ball-mill vessel equipped with steel balls (20g), the vessel was closed and the milling was carried out at room temperature at a speed of $25H_z$. After every 5minutes milling cycles, the progress of the reaction was monitored by thin layer chromathography. The milling continued for 20mins overall using TLC to monitor the reaction till no traces of reactant were found.

Characterization

Electronic absorption spectra of the metal complex were recorded as diffuse reflectance on a Lambda 35 UV/Vis spectrophotometer in the 200–900 nm range. The background spectrum of the glass plate employed was subtracted using the instrument's software. Infrared (IR) spectra of the solid compounds were recorded by the reflectance method on a PerkinElmer Spectrum 100 FT-IR system. Proton and 13C nuclear magnetic resonance (NMR) spectra were recorded in DMSO-d6 on a Jeol machine operating at 270 MHz and a Delta machine operating at 400 MHz, respectively, at 298 K. NMR tubes with a 5-mm internal diameter were used for all experiments. Tetramethylsilane (TMS) and the residual protonated solvent peak (DMSO-d6 at 2.5 ppm) were used to calibrate the chemical shift. Powder X-ray diffraction (PXRD) spectra of the compound were recorded on a Bruker D8 Avance machine, with CuK radiation. The PXRD spectra of the free ligand (pure drug) were compared with those of the complex. C, H, and N analyses were carried out using a Eurovector EA 3000 elemental analyzer. The melting/decomposition point of the complex was determined using a GallenKamp melting point apparatus. Thermal analysis was carried out by the use of a TGA Q 500 V6.7 build 203 instrument in thermogravimetry (TGA) mode from ambient to 500°C. Solubility of the complex was determined in hot and cold water, *N*,*N*-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and other common organic solvents by shaking a small amount of compound in the solvent in a test tube. The mass spectrum of the complex was recorded on a Q Star mass spectrometer using nano-electrospray ionization (ESI)-expansion technique.

Experimental Design

Anti-malarial study was investigated in mice. The rats were supplied with normal rat chow and water *ad libitum*. The experimental animals were randomly divided into six groups as follows:
Group 1- not treated not infected (control)
Group 2- infected and not treated
Group 3- infected and treated with standard drug
Group 4- infected and treated with pure Trimethoprim
Group 5- infected and treated with Trimethoprim-Cu complex
Group 6- not infected and treated with Trimethoprim-Cu complex
Group 1 consisted of uninfected mice and received

0.2ml distilled water. The mice in group 2 consisted of infected mice and also received 0.2ml distilled water. The drug solution (0.2ml) prepared with DMSO were administered orally to the infected mice in groups 3, 4 and 5. Equivalent of therapeutic dose, 7.7mg/kg body weight of drugs was administered daily for a period of 3 days. All the animals were sacrificed 24 hours after the last administration.

Inoculation and Confirmation of Malaria Parasite

The mice were screened for malarial parasites in their blood and only those that were free from malaria parasites were selected for this study. The selected mice were acclimatized for two weeks in the laboratory after which each mouse was induced with standard inoculums of 1 x 10^7 *Plasmodium berghei* via intra-peritoneal route. The parasite was then allowed to incubate in the mice for 72 hours after which thick blood film was prepared from tail snip, and stained using Giemsa stain and the parasitaemia confirmed by viewing using x100 objective of the light microscope. This method was also used for parasitaemia count throughout the period of study and percentage parasitaemia determined.

Toxicity evaluation

Enzyme assays

The procedure described by Roy (1970) was used to assay alkaline phosphatase (ALP) activity. Aspartate aminotranferase (AST) and alanine aminotranferase (ALT) activity was assayed using the method of Reitman and Frankel [22]. Lactate Dehydrogenase activity was measured following the method of Kubowitz and Ott[23] as modified by Wrobleski and La Due[24]. Acid phosphatase activity was measured as described by Wright *et al* [25].

Hematological Parameters

Packed cell volume (PCV), haemoglobin level (Hb), white blood cells count (WBC), mean corpuscular hemoglobin concentration (MCHC) and platelets count were determined using the methods of Adewuyi and Olatunji [25].

Statistical Analysis

All data are presented as mean ± standard deviation. The data from the various groups were compared for statistical significance using Duncan Multiple Range test according to [26]. In all cases, probability level of 95% was taken as significant.

Results

Curative Test

Percentage parasiteamia

The percentage parasitaemia on the 4th, 5th, 6th, and 7th day after inoculation is represented in Figure 1. Percentage parasite as expected was significantly low on day 0 after inoculation compared to the other days. From the 5th day, a decline in the percentage was observed while the lowest percentage was recorded on the 7th day aside the control group. There was significant increase ($p<0.05$) in parasitaemia in all the treated groups when compared to day 0 except on the 7th day (Figure 1). The parasite level in mice treated with chloroquine declined below 5% by the 5th day, while those treated with pure trimethoprim and trimethoprim copper complex, declined by 6%. The percentage reduction of parasitaemia in the standard drug (chloroquine) is comparable with that of trimethoprim (pure and copper complex) groups. Mice treated with pure and trimethoprim-copper complex showed significant reduction ($p>0.05$) in parasite level. However, all the animals in all the groups survived beyond the experimental period.

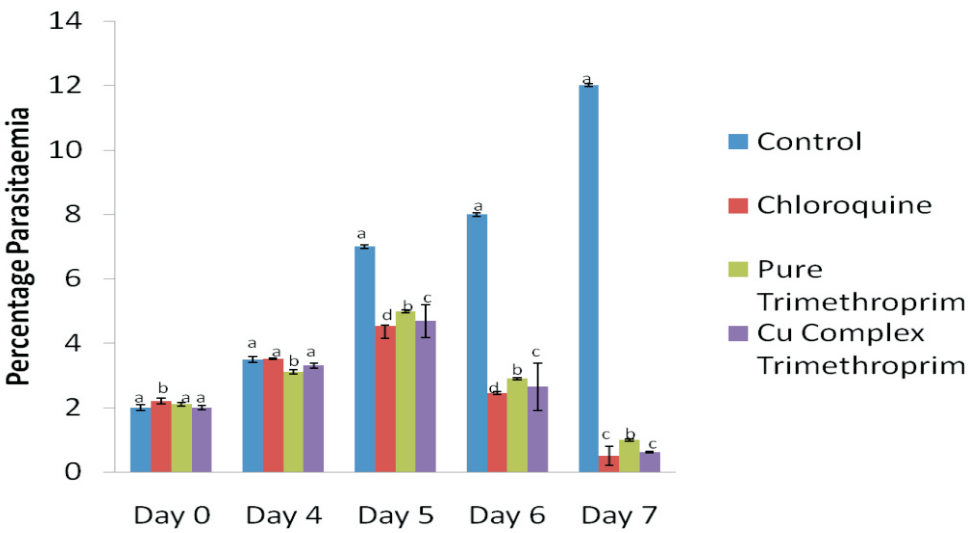

Figure 1: Percentage Parasitaemia in Infected Mice Treated with Trimethoprim and Trimethoprim Copper Complex

Table 2: Effect of Table 1: Percentage Chemo Suppression of Parasite Multiplication by the Drugs

Percentage chemo suppression of parasite multiplication of the drug

Treatments	Day 5	Day6	Day 7
Chloroquine	35.71	69.37	92.85
Trimethroprim	28.71	58.71	85.85
Trimethroprim-Cu Complex	33.00	62.28	91.14

Trimethoprim and Trimethoprim Copper Complex on the Specif ic Activity of Alkaline Phosphatase in selected Tissues and Plasma of Mice

	Control	IBNT	ITWC	ITWT	ITTC	NITC
Liver (µmol mL^{-1}min^{-1})	2.58 ± 0.21^{a}	1.31 ± 0.09^{b}	3.98 ± 0.15^{c}	5.78 ± 0.22^{d}	6.04 ± 0.44^{e}	2.12 ± 0.15^{a}
Kidney (µmol mL^{-1}min^{-1})	3.30 ± 0.17^{a}	3.09 ± 0.17^{a}	4.41 ± 0.29^{b}	3.62 ± 0.20^{a}	3.70 ± 0.57^{a}	11.09± 1.03^{d}
S.I (µmol mL^{-1}min^{-1})	4.31 ± 0.32^{a}	3.41 ± 0.22^{a}	5.59 ± 0.70^{a}	21.37± 1.05^{c}	19.55± 0.62^{d}	10.48± 0.48^{e}
Plasma (µmol mL^{-1}min^{-1})	15.75±6.74^{a}	15.50±4.33^{b}	13.75±5.63^{c}	14.75±7.97^{d}	14.85±5.73^{c}	14.50±6.56^{d}

Values are mean of 5 determinants ± SEM Rows with different superscript are significantly ($p < 0.05$) different.
IBNT = Infected but Not Treated
ITWC = Infected and Treated with Chloroquine
ITWT= Infected and Treated with Trimethoprim (pure)
ITTC = Infected and Treated with Trimethoprim-Cu Complex
NITC= Not Infected and treated with Trimethoprim-Cu Complex
S.I. = Small Intestine

Table 3: Effect of Trimethoprim and Trimethoprim Copper Complex on the Specific Activity of Acid Phosphatase in the liver, Kidney, Small Intestine and Plasma of Mice

	Control	IBNT	ITWC	ITWT	ITTC	NITC
Liver (µmol mL^{-1}min^{-1})	8.51±0.39^{a}	5.89± 0.15^{b}	11.18±1.10^{c}	10.96±1.01^{c}	10.53± 0.89^{c}	5.87 ± 0.39^{b}
Kidney (µmol mL^{-1}min^{-1})	0.96±0.03^{a}	1.90 ± 0.09^{b}	6.11±0.19^{c}	5.46 ± 0.39^{c}	7.51 ± 0.43^{d}	7.50 ± 0.68^{d}
S.I (µmol mL^{-1}min^{-1})	4.18±0.22^{a}	12.20± 0.51^{b}	12.90 ±0.75^{b}	13.93± 0.62^{c}	17.92± 0.98^{d}	13.11± 0.62^{c}
Plasma (µmol mL^{-1}min^{-1})	6.87±0.38^{a}	8.34 ±0.37^{b}	4.36±0.23^{c}	4.59±0.26^{c}	4.47±0.23^{c}	9.79 ±0.82^{d}

Values are mean of 5 determinants ± SEM Rows with different superscript are significantly ($p < 0.05$) different.

Table 4: Effect of Trimethoprim and Trimethoprim Copper Complex on the Activity of Alanine Aminotransferase in Selected Tissue and Plasma of Mice

	Control	IBNT	ITWC	ITWT	ITTC	NITC
Liver (µmol mL^{-1}min^{-1})	1.83±0.12^{a}	0.67±0.04^{b}	0.64 ± 0.03^{b}	0.52±0.08^{b}	6.60 ± 0.34^{c}	5.17 ± 0.23^{e}
Kidney (µmol mL^{-1}min^{-1})	13.63±1.02^{a}	7.93±0.76^{b}	4.68 ±0.22^{c}	7.32±0.31^{b}	16.00 ±0.88^{a}	10.32 ±0.62^{d}
S. I (µmol mL^{-1}min^{-1})	1.87±0.13^{a}	0.94±0.04^{b}	0.74 ± 0.02^{c}	0.32±0.02^{d}	0.89 ±0.02^{b}	0.63 ±0.03^{c}
Plasma (µmol mL^{-1}min^{-1})	15.50±1.12^{a}	9.75±0.53^{b}	11.50±1.07^{c}	8.25±0.94^{b}	11.75±1.06^{c}	16.00±1.25^{a}

Values are mean of 5 determinants ± SEM Rows with different superscript are significantly ($p < 0.05$) different.

Table 5: Effect of Trimethoprim and Trimethoprim Copper Complex on the Activity of Aspartate Aminotransferase in Selected Tissues and Plasma of Mice

	Control	IBNT	ITWC	ITWT	ITTC	NITC
Liver (µmol mL^{-1}min^{-1})	1.41 ± 0.10 ^{a}	1.10 ± 0.08^{a}	0.10 ± 0.01^{b}	0.54±0.03^{c}	0.67±0.05^{d}	0.69±0.03^{d}
Plasma (µmol mL^{-1}min^{-1})	55.23±3.34 ^{a}	53.89±3.26 ^{a}	44.50±1.13 ^{b}	41.75±2.35^{b}	47.75±3.75^{c}	66.75±3.76^{d}
Kidney (µmol mL^{-1}min^{-1})	2.86 ±0.24^{a}	1.62 ±0.11^{b}	0.63 ±0.04^{c}	0.56±0.02^{c}	0.23±0.01^{d}	1.23±0.08^{b}
S.I. (µmol mL^{-1}min^{-1})	0.83 ± 0.01^{a}	0.46 ± 0.02^{b}	0.64 ±0.03^{c}	0.84±0.05^{a}	0.38±0.02^{d}	0.53±0.02^{e}

Values are mean of 5 determinants ± SEM Rows with different superscr ipt are significantly ($p < 0.05$) different.

Table 6: Effect of Trimethoprim and Trimet hoprim Copper Complex on the Activity of Lactate Dehydrogenase in the Liver, Kidney, Small Intestine and Plasma of Mice

	Control	IBNT	ITWC	ITWT	ITTC	NITC
Liver (µmol mL^{-1}min^{-1})	6.84 ±0.52^{a}	8.29 ±0.29^{b}	4.83 ±0.23^{c}	3.24 ±0.27^{c}	3.86±0.18^{d}	7.94±0.56^{b}
Kidney (µmol mL^{-1}min^{-1})	12.42 ±1.13^{a}	11.89 ±0.92^{a}	3.57±0.29^{b}	8.87 ±0.43^{c}	6.16±0.46^{d}	10.15±0.71^{e}
S.I (µmol mL^{-1}min^{-1})	1.45±0.09^{a}	11.83±1.06^{b}	7.54±0.43^{c}	8.82±0.71^{c}	6.15±0.32^{d}	3.01±0.04^{e}
Plasma (µmol mL^{-1}min^{-1})	12.19 ±1.02^{a}	6.81 ±0.42^{b}	19.72±0.76^{c}	21.36±1.67^{d}	19.36±1.63^{c}	13.14±1.16^{a}

Values are mean of 5 determinants ± SEM Rows with different s uperscript are significantly ($p< 0.05$) different.

Table 7 : Haematological Parameters of Mice Administered with Trimethoprim and Trimethoprim - Copper Complex

	Control	IBNT	ITWC	ITWT	ITTC	NITC
PCV (%)	40.25±1.89^{a}	34.50±1.55^{b}	37.25±1.25^{c}	32.75±3.33^{b}	33.00±1.68^{b}	39.50±0.50^{a}
Hb (g/dL)	13.27± 0.57^{a}	11.40±0.14^{b}	12.35±0.63^{c}	10.08±0.93^{d}	10.20±0.46^{d}	12.28±0.14^{c}
RBC (g/L)	4.66±0.27^{a}	3.79±0.83^{b}	4.56±0.24^{a}	3.36±0.31^{b}	3.44±0.80^{b}	3.89±0.09^{b}
MCV (fl)	80.00±3.89^{a}	87.75±0.63^{b}	78.75±3.84^{a}	75.75±4.71^{c}	80.25±2.78^{a}	88.50±2.25^{b}
MCH (pg)	26.75±2.14^{a}	29.00±1.73^{b}	27.25±1.11^{a}	24.50±1.44^{c}	25.50±0.87^{c}	29.00±0.41^{b}
MCHC (g/dL)	31.25±0.85^{a}	31.00±0.41^{a}	31.75±0.85^{a}	30.75±0.48^{a}	30.75±0.25^{a}	31.25±0.63^{a}
WBC (g/L)	3.68±0.50^{a}	5.23±0.17^{b}	4.00±0.32^{a}	6.40±0.74^{c}	6.60±1.17^{c}	6.58±0.31^{c}
NEUTROPHIL(%)	22.00±1.68^{a}	32.75±3.01^{b}	44.75±3.35^{c}	44.75±3.35^{c}	30.50±3.30^{d}	37.75±2.39^{e}
LYMPHOCYTE(%)	78.00±1.68^{a}	67.25±3.01^{b}	55.25±3.35^{c}	66.25±2.59^{b}	69.50±3.30^{b}	62.25±2.39^{d}

Values are mean of 5 determinants ± SEM Rows with different superscript are significantly ($p < 0.05$) different

Percentage Chemo-suppression

The percentage chemo-suppression in the groups treated with trimethoprim (pure and copper complex) are relatively similar to that of the chloroquine group which increased significantly ($p<0.05$). Pure trimethoprim attained 85.85% by day 7 while trimethoprim copper complex attained 91.14% (Table 1).

Toxicity Evaluation

The effects of administration of Chloroquine, Trimethoprim and Trimethroprim-copper complex in mice liver, kidney and small intestine on alkaline phosphatase, acid phosphatase, Alanine amino transferase, aspartate amino transferase and lactate dehydrogenase activities are shown in Table 2, 3, 4, 5 and 6 respectively.

Administration of the drugs (Chloroquine, Trimethoprim and Trimethoprim-copper complex) in all the experimental groups, with mice infected and treated, produced a significant increase ($p < 0.05$) in alkaline phosphatase activities of mice liver, kidney and intestine when compared with the control group. There was no increase in plasma ALP activities in the parent drugs and copper-derivative when compared with the control. Acid phosphatase (ACP) concentration in the kidney and small intestine was significantly higher ($p<0.05$) across all the groups when compared with Control group, whereas, in the liver, only in the groups of malaria-infected animals

treated with chloroquine, trimethoprim and trimethoprim complex was a significant increase ($p<0.05$) observed while other groups produced a significant decrease ($p>0.05$), when compared with the control group. Also, the drug resulted in significant decrease ($p<0.05$) in the activity of acid phosphatase in the plasma of animals, when compared to mice in the control group (Table 3).

The activity of alanine aminotransferase (ALT) in the liver was significantly decreased ($p<0.05$) across all the groups, except in the mice infected and treated with trimethoprim-copper complex and uninfected animals treated with trimethoprim-copper complex in which a significant increase ($p<0.05$) was observed when compared with the Control group. In the kidney, a significant decrease ($p<0.05$) in ALT activity was observed across the groups except group of infected animals treated with the trimethoprim-complex, whereas, in the small intestine, a significant decrease ($p<0.05$) in ALT activity was observed across the groups. The activity of ALT in the plasma was significantly decreased ($p<0.05$) across all the groups, except in trimethoprim-complex treated uninfected group where no significant alteration was observed when compared to mice in the control group (Table 4).

Aspartate aminotransferase

A significant decrease ($p<0.05$) in concentration of aspartate aminotransferase (AST) was observed in the liver (except in group with infected and untreated animals, where no significant alteration was observed when compared to mice in the control group), kidney and small intestine across all the groups when compared with the control group (Table 5). In the plasma of the animals, there was also a significant decrease ($p<0.05$) across all the groups, except in the group containing uninfected mice treated with trimethoprim-complex, in which a significant increase ($p<0.05$) was observed and the infected and untreated group which showed no significant difference($p>0.05$) from the control.

Lactate dehydrogenase

In all the groups, a significant decrease ($p<0.05$) in the activity of lactate dehydrogenase (LDH) was observed in the liver and kidney except in the liver of the group of mice infected but not treated where a significant increase ($p<0.05$) was obtained and the kidney of the same group in which no significant alteration($p>0.05$) was observed when compared with the Control group. There was a significant increase in the activity of lactate dehydrogenase (LDH) in the small intestine ($p<0.05$) across all the groups when compared with the Control group. In the plasma of the animals, there was a significant increase ($p<0.05$) across all the groups, except in mice in the trimethoprim-copper complex treated uninfected group which produced no significant alteration ($p>0.05$) when compared to mice in the control group and the group of mice infected but not treated, in which a significant decrease ($p<0.05$) was observed (Table 6). There was a significant decrease ($p<0.05$) in the levels of PCV, haemoglobin, RBC and lymphocytes across all the groups, when compared with the Control group except the PCV of group of mice not infected but treated with Trimethoprim-Cu complex. Conversely, there was a significant increase ($p<0.05$) in the levels of WBC and neutrophil concentrations across the treatment groups when compared with the Control group. The concentrations of MCHC do not produce any significant alteration ($p>0.05$) across the test groups. The malaria-infected mice treated with the reference drug (chloroquine) and trimethoprim Cu-complex did not show any significant difference in the concentrations of MCV when compared with the control group (Table 9) but a significant increase ($p<00.5$) was observed in the malaria-infected untreated mice and trimethoprim Cu-complex treated uninfected mice when compared with the mice in the Control group. In the malaria-infected untreated mice and trimethoprim-complex treated uninfected mice, the concentrations of MCH in the plasma was significantly increased ($p<0.05$), whereas, the groups of malaria-infected animals treated with the trimethoprim and trimethoprim-Cu complex showed a significant reduction ($p<0.05$) while there was no significant alteration ($p>0.05$) in the concentration of MCH in the group of mice infected and treated with standard group (Chloroquine) when compared with mice in the control group.

Discussion

Plasmodium berghei has proved to be very convenient for the detection of antimalarial activity and it is generally considered that the infection with this parasite in the mouse is a valid model for the primary screening of conventional drugs for eventual use against human malaria [27]. At initiation of malarial infection, the parasite infects erythrocytes and towards the peak of infection, invades predominantly more reticulocytes. At this stage, the animal becomes anaemic, with remarkable increase in the rate of destruction of red blood cell, which eventually triggers the erythroid tissue to start producing more reticulocytes to compensate for the destroyed erythrocytes [28]. The result of this study showed a progressive increase in the level of parasitaemia as the days (duration) progressed from day 3 to 7 in the infected mice (Figure 1). This is in line with the view that parasitaemia increases progressively after inoculation or infection until the point of death in the absence of suitable treatment [29,30].

The *in vivo* antiplasmodial activity of trimethoprim-copper complex showed that the

complex is more efficient in parasite clearance than trimethoprim, however trimethoprim-copper complex showed curative antimalarial activity comparable to chloroquine but not as effective as chloroquine. Thus, coordination to the metal enhanced the activity of the drugs. This could be due to the complexes binding first without being decomposed at the receptor site and also the deposition of free metal ions in the membrane of the parasites. This finding is in agreement with results of the studies carried out by Biot *et al.* [31] where they reported that incorporation of a metal ion into a molecule resulted into formation of compound with enhanced activity. The antiplasmodial activity of trimethoprim-copper complex in this study is higher when compared with the antiplasmodial activities of trimethoprim-cobalt and cadmium complexes in studies carried out by Tella and Obaleye [32]. Also trimethoprim-copper complex showed a greater antiplasmodial activity than the parent drug when compared with the antiplasmodial activities of trimethoprim-cobalt and cadmium complexes as observed in previous studies carried out by Tella and Obaleye [32]. This indicates a better enhancement of activity by derivatizing with copper.

Alkaline phosphatase and acid phosphatase are majorly used as marker enzymes for plasma membrane derangement. Alkaline phosphatase is employed to assess the integrity of plasma membrane and endoplasmic reticulum [33, 34]. The observed increase in ALP activities in the liver, kidney and intestine of mice infected and treated with the parent drugs and copper-derivative of trimethoprim suggests stimulation of the enzyme activities by the drugs and their metabolites which could be due to stress imposed on the tissues by the drugs [34]. Alkaline phosphatase is a marker of cholestatic complications which may arise from hepatic parenchymatous tissue. Thus the complex exhibited lower toxicity than the parent drugs. This finding is in agreement with previous work of Biot *et al.*[35]; O. Neil *et al.*[36]; Zhou *et al.* [37]; Tella and Obaleye [32].

Acid phosphatase (ACP) is generally known as a 'marker' enzyme for the lysosomal membrane [38]. The increase in the activity of *ACP observed in the* kidney, liver and small intestine of the infected and treated groups (with the largest elevation occurring in the infected group treated with trimethropim-Cu complex) may be adduced to an increase in functional activity of the organ, due to interaction of drugs and the plasmodium, leading to *de novo* synthesis of the enzyme molecule *in situ* [39]. This could result in indiscriminate hydrolysis of phosphate esters, which are potential energy source for the cell [40]. This may suggest a possible threat to the well-being of the organs as this may result in autolysis and consequently cell death [41].

Aminotranferases are marker enzymes for liver cytolysis and can be used to ascertain any kind of damage to the liver [42]. Decrease in the activity of alanine aminotransferase and aspartate aminotranferase in the selected tissues of mice in the infected groups treated with the drugs and a corresponding decrease in the activity of these enzymes in the plasma might be an indication that there was inactivation or inhibition of these enzymes in the tissues [39].

Lactate dehydrogenase is an enzyme localized in liver peroxisomes, muscles, kidney, heart and the soluble portion of the cell (cytosol) [43]. Therefore, the decrease in the lactate dehydrogenase activity of the liver and kidney and a corresponding increase in the plasma of the animals may be attributed to the leakage of the enzyme from the kidney and liver, probably due to disruption of the ordered bilayer membrane [44,39]. Thus, it may be inferred that the drugs play a role in lactate metabolism. This is in agreement with previous study by Chiagoziem *et al.* [45], in which a certain antimalarial (artesunate-amodiaquine combination) drug was indicted in playing a role in lactate metabolism.

Assessment of haematological parameters can be used to explain blood relating functions of chemical compounds [46]. Haematological parameters can also be used to investigate and determine blood diseases that affect the production of blood (haematopoiesis) and its components, such as PCV, RBC, WBC, neutrophils, lymphocytes etc. Reduction in haemoglobin concentration might be accompanied by a fall in the RBC and PCV [47]. The decrease in the RBC and PCV observed in groups of mice infected and treated might be due to decrease in the rate of production of erythrocytes. It may also be that the drugs lacked erythropoeitin potential [48]. Very low levels of RBC, haemoglobin and haematocrit predispose animals to anaemia [49]. This might be that the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues were reduced by the drug administration. The increase in WBC might be an indication that there was a challenge on the immune system. Increased level of neutrophil obtained may also indicate inflammatory response due to the toxic effect of the drugs on the tissue. Neutrophils are recruited to the site of injury within minutes following trauma and are the hallmark of acute inflammation (Cohen *et al.,* 2002). Therefore, the increase in neutrophil observed across the groups may result from the injurious effect of the administered drugs on tissues. The occurrence of low lymphocyte in the treated groups compared with the control is an indication that the drugs possibly inhibited T and B lymphocyte in mice through the blocking of amino acid uptake into the stimulated lymphocytes [50]. This may predispose the animals to overwhelming infections, cold, influenza or cancer [51]. A blood index, such as mean corpuscular haemoglobin concentration

(MCHC), is of importance in diagnosis of anaemia in most animals. Since, there was no significant change in MCHC of test groups; this may serve as an indication that there was no hypochromic anaemia or intra-cellular dehydration.

Conclusion

The study concluded that coordination of copper to trimethoprim increased its antimalarial activities. The biochemical parameters investigated in this study revealed functional and selective toxicity, therefore, trimethoprim-Cu may not be completely safe as oral remedy.

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Histomorphological Evaluation of Wound Healing - Comparison between Use of Honey and *Vernonia amygdalina* Leaf Juice

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Abstract

Vernonia amygdalina Del. crude sap has been used for wound management in ethnomedicine. Histological evaluation of *Vernonia amygdalina* leaf juice effect on wound healing was carried out. Female albino rats (150-200 g) were anaesthetized, shaved and induced a circular excision wound (10 mm diameter) at the dorso-lateral region. They were randomly assigned (n = 5) to honey, *Vernonia amygdalina* leaf juice and deionised water (negative control; NC) treated groups (topically treated twice/day with 20 µL each of the respective treatments). Wound size was measured using a digital camera (Canon Powershot 5.0MP, Canon, Tokyo, Japan) and Adobe photoshop CS5 software. Wound tissues were removed on days 3, 5, 7 and 10 post wounding for histomorphological examinations. Average time for complete wound closure in honey (11.00 ± 0.00 days) and *Vernonia amygdalina* leaf juice (11.20 ± 0.44 days) treated groups was significantly ($p<0.05$) shorter when compared to NC (13.4 ± 0.90 days). *Vernonia amygdalina* leaf juice, similar to honey, enhanced fibroblasts recruitment, epithelia cells migration, neovascularization and reduced polymorphonuclear leukocytes (PMNL) infiltration as compared to the NC at the early phase of wound healing (days 3 and 5). *Vernonia amygdalina* might enhance cutaneous wound healing through mechanisms associated with activation/differentiation of skin cells and inhibition of PMNL infiltration of wound area.

Keywords: Histology, *Vernonia amygdalina*, Honey, Wound healing.

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Introduction

Wound healing is a process involving series of events, which are categorized into phases: inflammation, proliferation/repair and remodeling. The inflammatory phase is characterized by blood clotting and inflammatory cell infiltration (inflammation). Proliferative phase is characterized by epithelial cells proliferation (epithelialization), fibroblasts and endothelial cells activation and migration (wound contraction and neovascularization) and collagen synthesis (collagenization). Replacement of type III collagen fibers with type I fibers and re-alignment of collagen fibers along tension lines by collagen cross linking are distinct features of remodeling phase[1, 2]. These specific events are not only complex but also fragile and susceptible to interruption or failure leading to formation of chronic non-healing wounds.

Millions of people around the world are suffering from cutaneous wounds at various extent caused by various reasons such as surgical operation, trauma through road accidents, burns, disease conditions, professional and household accidents. An estimated excess of US$25 billion is spent annually on treatment of chronic wounds and the burden is rapidly growing due to increasing healthcare costs, an aging population and a sharp rise in the incidence of diabetes and obesity worldwide. The annual wound care products market was estimated at $15.3 billion in 2010. An additional burden of wound healing is the problem of skin scarring, a $12 billion annual market[3]. The vast economic and social impact of wounds in our society calls for attention and resources to explore effective wound management products that are safe, accessible and affordable to the common people.

In recent times, there is increase interest in the use of natural products especially plants' products for nutritional and medicinal purposes to improve the health condition and performance of humans and animals. Medicinal plants are relatively safe and affordable sources of raw materials for the production of modern drugs and main ingredient used by the traditional medical practitioners[4].

Natural honey is most commonly used natural

wound healing agent. It is composed of glucose, fructose, water, amino acids, vitamins, minerals, and other substances. Honey has antibacterial activity that helps to effectively prevent and clear wound infections. It has been reported for treating burns, leg ulcers, pressure ulcers and many more due to its antioxidant, antimicrobial, and anti-inflammatory activities. The chemical composition of honey and hence its bioactivity/efficacy was shown to depend on the location and time of collection and plant source. These factors alter H_2O_2, phenolic compounds contents, pH and osmotic pressure of honey which play roles in wound healing property. Honey, produced by honey bees from plant nectars, is relatively available and commonly collected from trees in farm yards, forest or apiary[5]. *V. amydalina*, A fodder tree, commonly grown in vegetable garden at homes is also beneficial in the management of wound and could complement the used of honey in wound healing as it is more accessible.

Vernonia amygdalina DEL (*V. amygdalina*), a member of the Asteraceae family, was reportedly the most used medicinal plant in the genus *Vernoniae*[6]. It is a common homestead farming vegetable and fodder tree commonly used in foods for their nutritional and medicinal benefits. The leaves are consumed either as a vegetable (macerated leaves in soup) in Nigerian soup (Ogbono soup) or Cameroon (Ndole) dish after removal of its bitter taste through soaking in several changes of water or by boiling in excess of water[7, 8]. A freeze dried sample of the extract of *V. amygdalina* has been formulated into tablets to boast the immunity of HIV patients[9] and control diabetes mellitus[10]. Biomass root culture extracts of *V. amygdalina* killed abnormal cells among primary cells harvested from patients with acute lymphoblastic leukemia and acute myeloid leukemia[11]. Ethnobotanical survey in the East and West African countries listed *V. amygdalina* as one of the most important herb for the management of wound in traditional medical practice[5].

Vernonia amygdalina phytochemicals and bioactive components have been duly considered and several compounds especially flavonoids and terpenoids have been isolated from the plant. A good number of the compounds e.g. vernolide and water soluble peptides (edotides) that were isolated from *V. amygdalina* exhibited cytotoxicity against cancer cell lines[12, 13]. Fifteen compounds that were detected from other *Vernonia* genus have also been isolated from *V. amygdalina* and they were biologically active in various assays. Octahydrovernodalin is the most important bitter-principle in the plant resulting in the common name of "bitter leaf" for the plant[14]. The antimicrobial, anti-hyperglycemic, anti-inflammatory, analgesic and anti-oxidant properties of *V. amygdalina* which were previously reported suggest its potential benefit in the management of wound[14]. Despite these beneficial properties of *V. amygdalina* in wound, few studies reported it effect on the rate of wound closure and the histomorphological examination that could give an insight to the restorative and reparative processes due to *V.amygdalina* application during wound healing was not reported. Therefore, this study was planned to determine histomorphologically how topical treatment of cutaneous excision wound with *V. amygdalina* enhanced wound repair processes as compared to honey using a rodent model.

Materials and methods

Plant material

Fresh leaf of *V. amygdalina* (bitter leaf), was collected from a farm at Oke-Ose, Ilorin, Kwara state, Nigeria and identified by S.B. Adeyemi of the Department of Plant Biology Herbarium, University of Ilorin, Ilorin, Nigeria. The voucher specimen was deposited at the herbarium (Specimen number: UIH003/475).

Collection of fresh V. amygdalina leaf juice

The fresh leaf was washed thoroughly 3 times with running tap water and air dried in room temperature. The fresh leaf was then blended, squeezed vigorously and filtered using a fine muslin cloth (mesh cloth) in order to obtain a fresh concentrated bitter leaf juice that was used in this study. There was no water/moisture or solvent added to the fresh concentrated *V. amygdalina leaf juice*. The juice is therefore 100% concentrated and 4% by weight yield was obtained when 70 g initial weight of the fresh *V. amygdalina* leaf was processed as explained in this section to obtain the juice. The fresh *V. amygdalina* leaf juice (20 µL) was used for the topical treatment of the excision wound.

Collection of honey

Unilorin multifloral bee honey, produced by Apis mallifera adansonii Latreille was used in this study. The honey which was reportedly produced from 13 plant species belonging to 13 different families was collected from Unilorin Apiary Farm in June, 2015[15]. The pure honey (20 µL) was used for the topical

treatment of rats' excision wound.

Animals

Female albino rats weighing 150-200 g, purchased from a vendor at No. 3, Baba Ibo Street, Asa-Dam, Ilorin, were used in this study. They were housed in the Faculty of Basic Medical Science, University of Ilorin animals' house under standard housing conditions throughout the experiment. The animals were left for seven days at room conditions for acclimatization. Animals were randomly selected in groups of five per cage, fed with standard rats' chow and filtered tap water *ad libitum*.

Experimental design

Rats were randomly assigned to three groups of 25 rats each: the negative control groups (NC) were treated with de-ionised water, the honey group (H) were treated with pure Unilorin honey and V. amygdalina group (VA) were treated with fresh undiluted *V. amygdalina* leaf juice. The respective treatments were topically applied on the excision wound twice/day with 20 µL each. *A*nimals showing any sign of skin infection prior to the commencement of the experimental procedure were excluded from the study and replaced with animals that have intact skin. Five rats from each group i.e. n = 5 were observed until complete wound closure (CWC) and the remaining 20 rats from each experimental group were sacrificed in order of five animals i.e. n = 5 on days 3, 5, 7 and 10 post wounding to obtain the healing skin sample.

Animal ethical committee approval

All handling and management procedure were carried out in accordance with the guideline for care and use of laboratory animals of the University of Ilorin. The protocol, experimental design and animal handling procedure were approved by the Ethical Committee, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.

Induction of excision wound

Rats were anaesthetized with hydrochloride ketamine (40 mg/kg i.m) on the thigh region prior to wound induction. The dorso-lateral fur of the rats' dorsum was shaved using an electric clipper (Chaoba, Japan) and the shaved region was marked with methylene blue using a clean circular plastic stencil of 10 mm diameter. A full thickness excision wound of circular area of about 160 mm^2 and 2 mm depth was created along the mark using forceps, scalpel and scissors.

Evaluation of wound closure

Change in wound size (surface area) was monitored and recorded using a digital camera (Canon powershot A495: Canon, Inc.) daily. The Photograph of wound area was evaluated using Adobe photoshop CS5 Extended (Version 12.0.4 x 32: Adobe, Inc.) following the method previously described[15]. The percentage reduction in wound size was calculated using the equation 1.

$$\%\,\text{Wound size reduction} = \frac{SA1 - SAD}{SA1} \times 100 \quad \ldots\ldots\ldots\ldots 1$$

SA1; initial wound surface area, SAD; wound surface area at different days.

Percent relative wound healing efficiency (%RWHE) of honey and *V. amygdalina* leaf juice were calculated to compare the rate of healing by the test agents with the rate of healing without treatment (natural healing). %RWHE was calculated using equation 2 according to Abdulrazaq et al., (2013)[16].

$$\%\,RWHE = \frac{TN - TE}{TN} \times 100 \quad \ldots\ldots\ldots\ldots 2$$

TN; time required for natural wound healing i.e., CWC with de-ionised water treatment and TE; the time (number of days) taken to attain CWC for the honey or *V. amygdalina* leaf juice treated groups.

Collection of wound tissue sample

At the selected days post wounding (days 3, 5, 7 and 10 post wounding), five rats from each group were euthanized using deep ether anaesthesia method. Full thickness wound tissue sample was carefully excised from the rats using scissors and forceps to remove sufficient amount of the surrounding wound margin. The wound area was cut deep into the underlying tissue to completely remove the granulation tissue from the healing skin of rats and was processed for histological studies.

Histological evaluation

The collected wound tissue was fixed in 10% formalin for 12 h, processed in graded ethanol concentrations, cleared and embedded in wax. Wound tissue sections (5 µm) were prepared using rotary microtome (Leica, Biosystems), mounted onto Superfrost Plus slides (Fisher Scientific) and stained in hematoxylin-eosin stain (H & E) following standard protocol. Histological examination was performed

under low power field (10× Magnification) using Nikon light microscope (Nikon, Tokyo, Japan) with digital camera attached to the ocular lens. Histological processes and structures such as re-epithelialization, PMNL (polymorphonuclear leukocytes infiltration), fibroblasts recruitment, neovascularisation (new blood vessels) and collagenization (collagen synthesis) were evaluated on 5 sections from each group according to the scale: 0, 1, 2, 3 and 4 by two independent observers who were blind to the experimental design[16]. The mean value was used for statistical comparison.

Semi quantitative scoring method

A modified semiquantitative scoring method of Gal et al. (2008)[17] was performed on the wound tissue sections based on 5 point scale.

Re-epithelialization was scored thus: 0; proliferating epithelial cells form thick blocks at the cut edge of the wound with little or no epithelial "tongue" protruding from the edge, 1; migrating epithelial cells protrude from the cut edge and cover less than 50% of the gap between the cut edges of the epidermis, 2; migrating epithelial cells cover at least 50% of the gap between the cut edges of the epidermis, 3; the migrating epithelial cells cover the wound surface completely, 4; presence of keratin on the new epithelial layer formed over the healing wound surface.

Similarly, PMNL infiltration was estimated: 0; absence of PMNLs as observed morphologically, 1; mild presence of PMNLs in the surrounding tissues of the wound, 2; mild presence of PMNLs in the granulation tissue and along the epithelial edge, 3; moderate presence of PMNLs in the granulation tissue and along the epithelial edge, 4; marked presence of PMNLs in the granulation tissue and along the epithelial edge.

Fibroblasts recruitment was also estimated on the 5 point scale: 0; absence of fibroblasts as observed morphologically, 1; mild presence of fibroblasts in the surrounding tissues of the wound, 2; mild presence of fibroblasts in the granulation tissue, 3; moderate presence of fibroblasts in the granulation tissue, 4; marked presence of fibroblasts in the granulation tissue.

Likewise, neovascularisation was estimated: 0; absence of new blood vessels as observed morphologically, 1; mild presence of new blood vessels in the subcutaneous tissue of the wound, 2; mild presence of new blood vessels in the granulation tissue, 3; moderate presence of new blood vessels in the granulation tissue, 4; marked presence of new blood vessels in the granulation tissue.

Finally, collagenization was evaluated: 0; absence of collagen fibres as observed morphologically, 1; presence of minimal amount of collagen fibres in the granulation tissue, 2; mild presence of collagen fibres in the granulation tissue, 3; moderate presence of collagen fibres in the granulation tissue, 4; marked presence of collagen fibres in the granulation tissue.

Statistical analysis

The statistical analysis was carried out using the software IBM SPSS version 20, and the data was expressed as mean ± SEM. ONE-way ANOVA, followed by Tukey's post-hoc test was used to analyse the data in order to obtain mean differences between the groups at different time intervals and $p<0.05$ was considered statistically significant.

Results

V. amygdalina accelerates wound healing similar to honey

The wound size showed no difference among the groups and the calculated percentage wound size reduction was 0% on day 0 when the wound was first inflicted for all groups (Figure 1A & 1B). Honey and *V. amygdalina* reduced the wound sizes significantly ($p<0.05$) and the percentage wound sizes reduction were 33.46 ± 0.37% and 30.50 ± 0.28% respectively on day 2 post wounding as compared to NC. However, on day 4 post wounding, honey alone reduced the wound size significantly ($p<0.05$) with 58.56 ± 0.38% reduction. From day 6 to day 12 post wounding, both honey and *V. amygdalina* similarly reduced significantly ($p<0.05$) the wound size which reached 100% reduction (complete wound closure; CWC) on day 12 post wounding when compared to NC. On day 12, average wound closure was 100% with honey and VA treated rats while it was ~ 93% for NC. NC exhibited complete wound closure after 2 weeks of treatment (Figure 1B). Average time for CWC was significantly ($p<0.05$) reduced compared to NC in the honey and *V. amygdalina* treated groups i.e. honey (11.0 ± 0.00 d) and *V. amygdalina* (11.2 ± 0.45 d) compared to NC (13.4 ± 0.90 d). The results showed that wounds were more rapidly healed in honey and *V. amygdalina* leave juice treated groups than the negative control. Most efficient healing as calculated from the equation 2, was observed with the honey (18%) and *V. amygdalina*

(16%) (Table 1).

V. amygdalina improved cutaneous wound histomorphology similar to honey

On day 3 post wounding, PMNL infiltration and fibroblasts recruitment were significantly ($p< 0.05$) reduced and increased respectively in honey and *V. amygdalina* treated groups when compared to NC semi quantitatively (Figure 2B & 2C). Honey alone, significantly ($p< 0.05$) increased the microvessels in the granulation tissue compared to NC (Figure 2D). However, traces of collagen deposit and little protrusion of the epidermis were observed in all groups. Scab consisting of necrotic tissues, blood clots rich in PMNL formed over the dermis of NC (Figures 3 & 4).

Honey and *V. amygdalina* significantly ($p< 0.05$) increased epithelialization, fibroblasts recruitment and microvessels and reduced PMNL infiltration compared to NC on day 5 (Figure 2A-D). Slight increased collagen deposit in *V. amygdalina* treated and less than 50% epidermal coverage of the wound surface were observed in the NC (Figures 3 & 4). Most of the Honey and *V. amygdalina* treated showed about 50% epidermal coverage of the wound (Figures 2A & 4). While there was no statistical significant difference among the groups on day 7 post wounding using semi quantitative score, increased microvessels in the NC group, marked epidermal closure of wound surface (> 50%) and moderately increased collagen deposit in the granulation tissue was observed in Honey and *V. amygdalina* treated group (Figures 2, 3 & 4).

The epidermal closure of the wound surface is completed with associated keratin layer observed in all groups on day 10. Honey led to thick epidermis and regenerating skin appendages. Moderate fibroblasts recruitment, collagen deposit, microvessels and minimal infiltrating PMNL were observed in all treated group and the semi quantitative comparison revealed no statistical significant difference among the groups for all the histological features observed on day 10 (Figures 2, 3 & 4).

Discussion

Many studies have reported the pharmacological properties of *V. amygdalina* which includes but not limited to antimicrobial, anti-hyperglycemic, anti-inflammatory, analgesic and anti-oxidant properties. These properties directly or indirectly prevent conditions that could potentially complicate the processes of wound healing. Bacterial bio-burden, nonenzymatic glycation of proteins, dysregulation of inflammatory and immune cells infiltration of wound, discomfort and oxidative tissue damage that are associated with impaired wound healing could be attenuated through the reported pharmacological properties of *V. amygdalina*[14].

Fresh leaf juice was used in the current study to mimic the ethnomedical practice. Usually, the fresh leaf of *Vernonia amygdalina* is macerated in water and the resulting water extract is drunk for most gastrointestinal disorder. As per the wound healing, the fresh leaf is forcefully squeezed to release the sap which is topically administered on wounds while the crushed leaf is used to cover the wound surface as a dressing material. The results showed that *V. amygdalina* fresh leaf juice accelerates excision wound healing by regulating the cellular activities, that affects the histological processes as observed at the selected early time points (days 3 and 5), in similar manner as honey.[18]

During the early phase of wound healing (at day 2), the *V. amygdalina* fresh leaf juice that was used in this study markedly reduced initial wound size by 30% which is comparable to honey (33% wound size reduction) (Figure 1B). The negative control reduced the wound size by 15.42% only on day 2. Honey, is a well established natural product which is commonly used in the clinics for the management of chronic wound[19, 20]. Since honey and *V. amygdalina* have similar percentage wound size reduction at the early phase, *V. amygdalina* could have properties similar to honey at the early phase of wound healing. The important event

Table 1. Average ti me for CWC and Wound healing efficiency

Treatment/group	CWC (days)	RWHE (%)
Negative control	13.40± 0.89	-
VA leaves juice	11.20±0.45*	16
Honey	11.00±0.00*	18

VA; *Vernonia amygdalina*, CWC; complete wound closure, RWHE; relative wound healing efficiency, * significant reduction at $p< 0.05$.

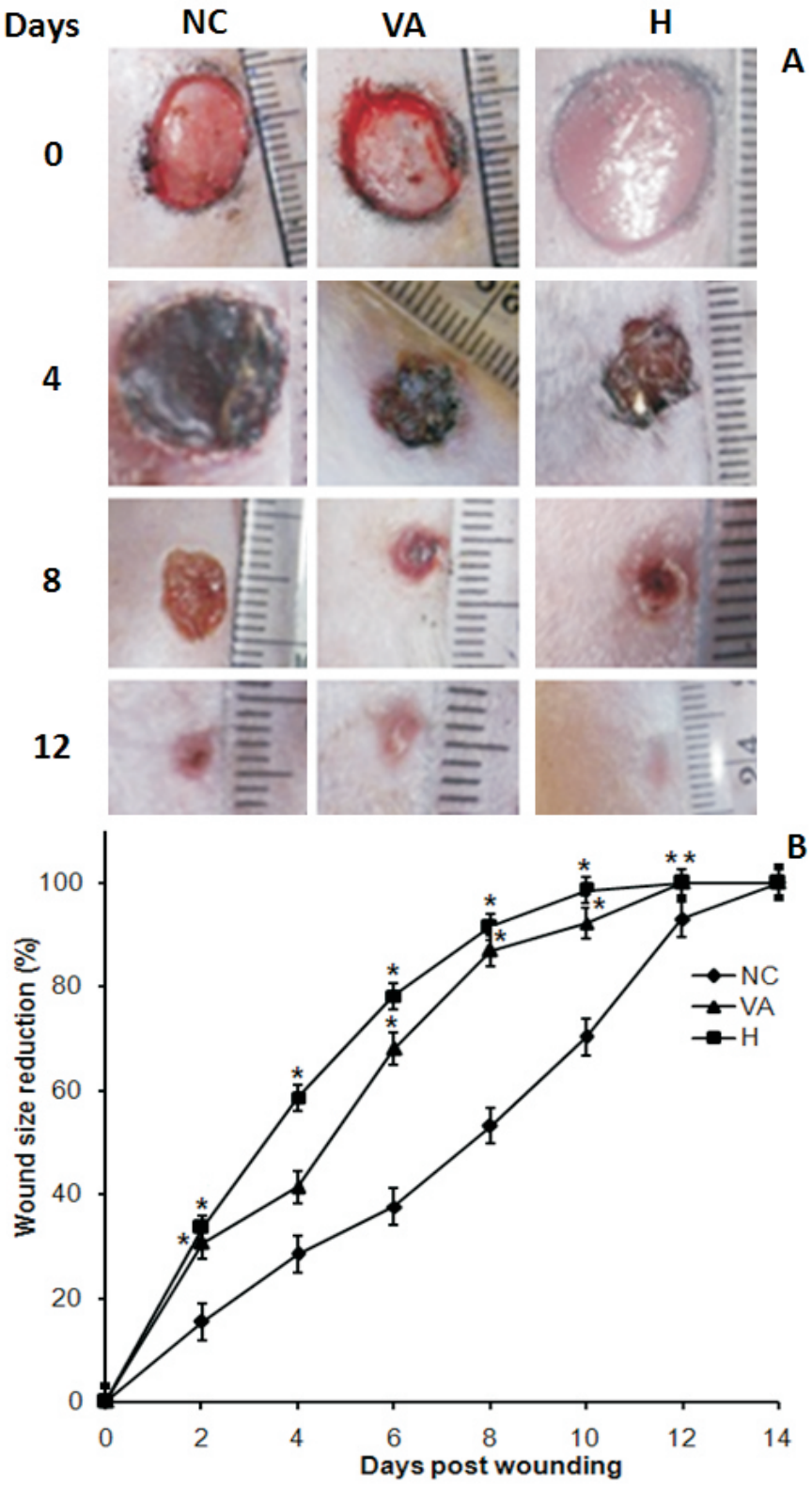

Figure 1. (A) Representative photographs of full-thickness excision wounds at different time intervals after topical application of honey (H) and *Vernonia amygdalina* leaves juice (VA) (20 µL) two times per day. (B) Rate of wound closure (% wound size reduction) in H and VA treated groups as compared to negative control. Wounds were more rapidly healed in H and VA treated groups than the negative control. Lines indicate mean ± SEM (n = 5). * indicates significantly ($p < 0.05$) reduced wound size as compared to NC.

of the wound healing cascade in the early phase is inflammation. Therefore, *V. amygdalina* might have induced healing early, due to it anti-inflammatory, antioxidant and antimicrobial properties, which were previously reported[14].

From day 6 to 12 post wounding: honey, and *V. amygdalina* comparably exhibited increased rate of healing *vs* negative control (Figure 1B). This shows that *V. amygdalina* did not only influence wound healing at the early phase but also enhanced healing cascades at all phases of wound healing. Recently, Eyo *et al*., (2014)[21] reported similar observation where they concluded that *V. amygdalina* proved more efficacious than other plant extracts that were tested in the study and that it is better for the treatment of all the phases of wound healing. However, the study subjectively evaluates the histomorphological changes and did not consider the quantitative histomorphological evaluation as performed in the current study.

Furthermore, the average time for CWC and the %RWHE for honey (11.00 ± 0.00; 18%) and *V. amygdalina* (11.20 ± 0.45; 16%) were notably improved compared to negative control (13.40 ± 0.89; 0%). Though, wound healing activity of honey is better but revealed no significant difference compared to *V. amygdalina*. These confirm the effectiveness of *V. amydalina* crude sap, the common form in which it is applied to wounds in traditional

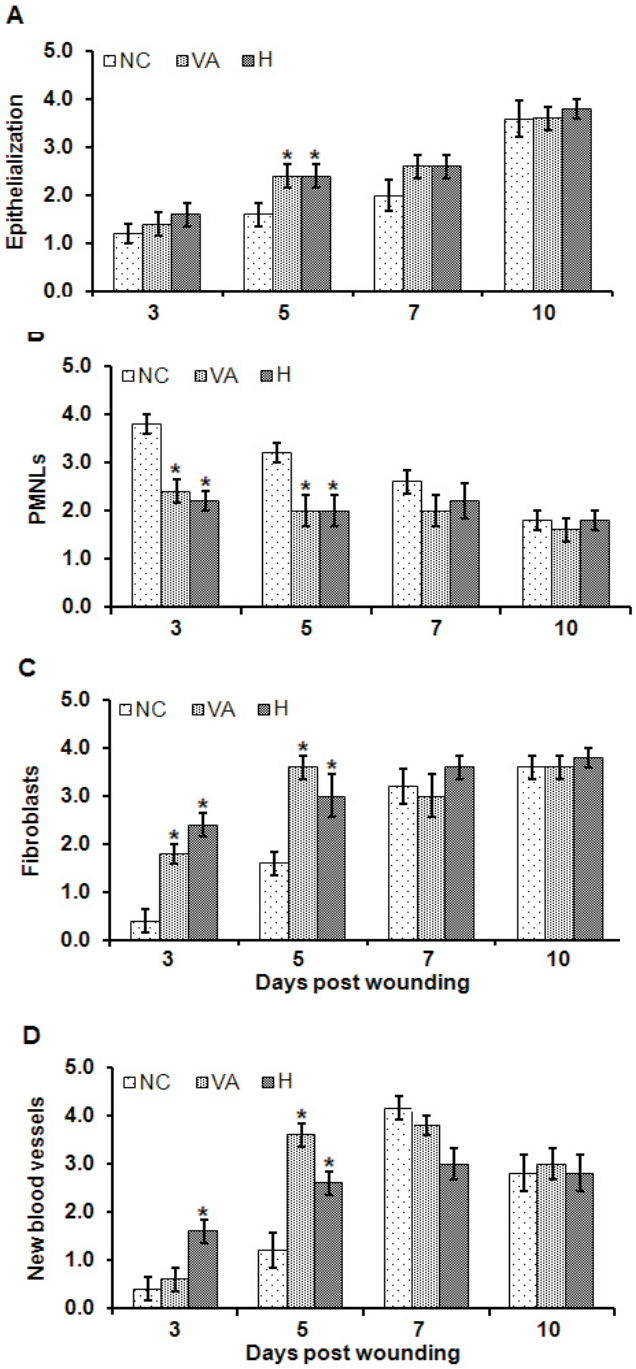

Figure 2. Semi quantitative assessment of histological structures/processes (epithelialization, polymorphonuclear lymphocytes infiltration, fibroblasts proliferation and neovascularization) in wound tissue sections after treatment with honey (H) and *Vernonia amygdalina* (VA) at the early (days 3 and 5) and later (days 7 and 10) phases of wound healing. Differences in the histological structures/processes among the groups were more noticeable at the early phase (days 3 and 5) of wound healing. There was no marked difference between H and VA treated groups however NC (negative control) differed significantly compared to both H and VA. Bars and lines represent mean ± SEM while * indicates significant ($p < 0.05$) difference as compared to NC.

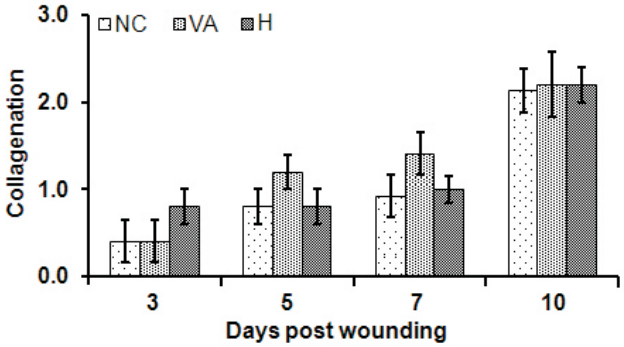

Figure 3. Semi quantitative assessment of collagen deposition (collagenization) in wound tissue sections after treatment with honey (H) and *Vernonia amygdalina* (VA) at the early (days 3 and 5) and later (days 7 and 10) phases of wound healing. There was no marked differences both at the early phase (days 3 and 5) and later phase (days 7 and 10) of wound healing. However, VA showed increase collagen deposition on days 5 and 7 while H exhibited increase collagen deposition on day 3 but the increased collagen deposition was not statistically significant. Bars and lines represent mean ± SEM while * indicates significant ($p < 0.05$) difference as compared to NC.

practice.

V. amygdalina is a plant with diverse medicinal potentials. Several classes of phytochemical constituents were found in it. Flavonoids, alkaloids, terpenes, saponins and sesquiterpenes[22] were reportedly found in *V. amygdalina*[23, 24] and steroids, coumarins, phenolic acids, lignans, xanthones and anthraquinone were also detected in *V. amygdalina* in another study[25]. It is classically known for its antimicrobial, anti-inflammatory and antioxidant properties[26]. Antioxidant, antimicrobial and anti-inflammatory properties of *V. amygdalina* were attributed to the presence of flavonoids, vernodalol, and tannins obtained from its extract respectively[27].

Histomorphological examination revealed marked increased fibroblasts, microvessels, epithelialization, reduced PMNLs and non significant change in collagen deposition in the early phase of wound healing (on days 3 & 5) in *V. amygdalina* and honey treated groups. Significant increased epithelialization was evident on day 3 in *V. amygdalina* and honey treated groups while honey alone produced pronounced increased microvessels on day 3 as compared to control using semi quantitative approach. Variation in the selected histological features and processes between treated and control groups in the later phase of wound healing (days 7 and 10) was not statistically significant (Figure 2, 3 & 4).

Few studies that have reported the potential of *V. amygdalina* on wound healing did not examine the histomorphological changes in wound following treatment with the plant's extract or the leave juice as used in the current study[19, 21, 28, 29]. Assessment of wound tissue histology could delineate the characteristic cellular mechanisms by which *V. amygdalina* enhanced wound repair at different phases of wound healing. From the foregoing observations, the observed increased fibroblasts recruitment, epithelia cells migration over the wound area, endothelial cells formation of microvessels and inhibition of excessive

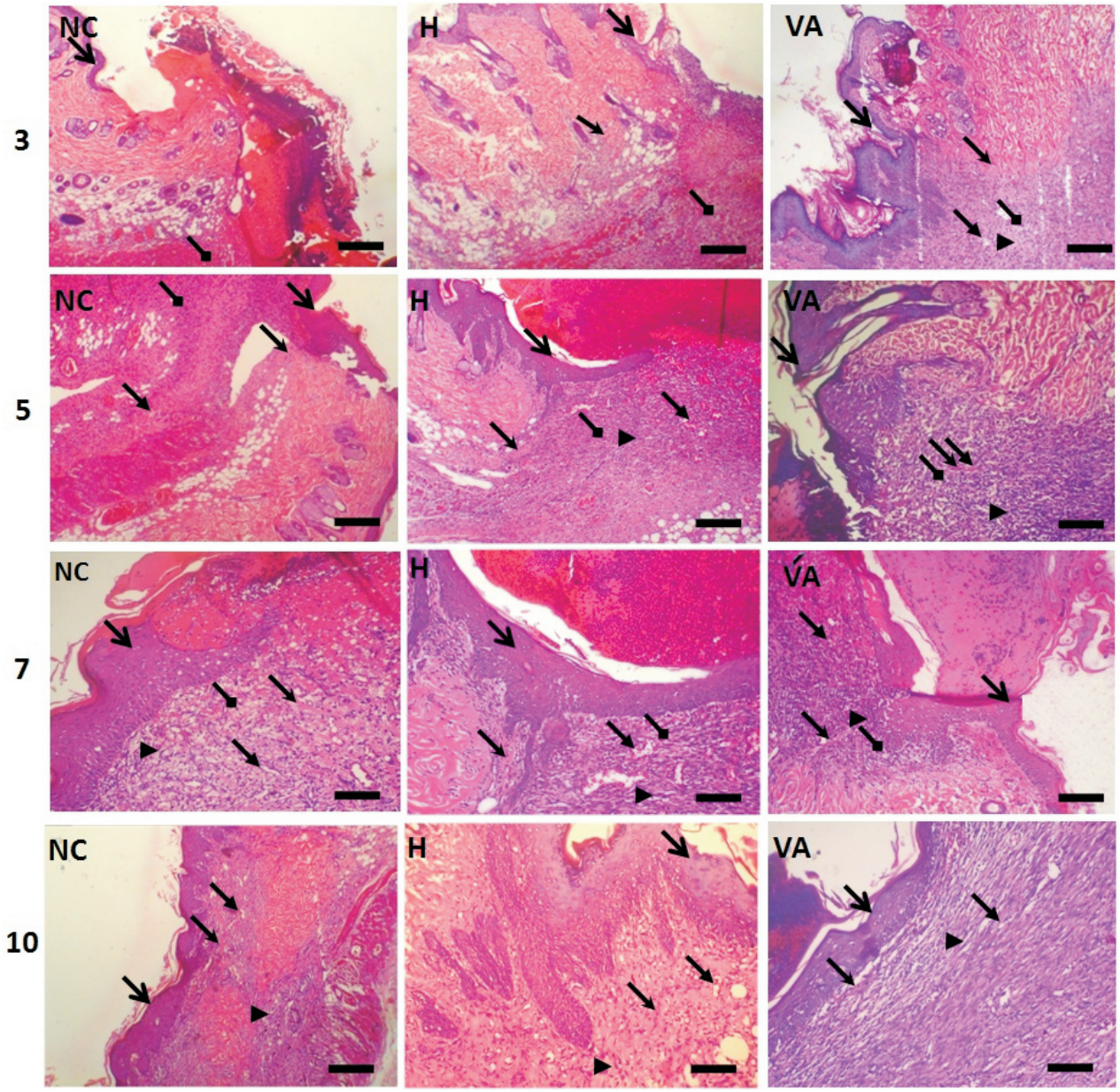

Figure 4. Representative photomicrographs of hematoxylin and eosin stained wound tissue sections showing [epithelialization (open arrow), polymorphonuclear leukocytes infiltration (Diamond arrow), fibroblasts proliferation (arrow head), neovascularization (arrow) and collagenization (stealth arrow)] on days 3, 5, 7, and 10 post wounding after treatment *with* Honey (H) and *Vernonia amygdalina* (VA). On days 3 and 5 post wounding, disrupted dermis, high density of infiltrating PMNLs and scab/necrotic tissues covering the wound were observed in the NC wounds. Wounds treated with H and VA showed reduced number of infiltrating PMNLs with increased number of fibroblasts as compared to NC. High density of microvessels, and extensive epithelialisation were observed in H and VA treated groups on day 7 post wounding. High density of fibroblasts, thicker epidermal layer and increased collagenization were observed in H and VA treated groups on day 10 post wounding. NC exhibited thinner epidermal layer on day 10 post wounding. Scale bars = 100 µm.

PMNL infiltration of wound area, as compared to the control, sufficiently showed potential role of *V. amygdalina* on cellular activities during cutaneous wound repair. Therefore, it is clear that *V. amygdalina* enhanced cutaneous wound repair via activation, migration, proliferation and differentiation of different cells of the skin. The semi quantitative comparison used, in the current study, revealed slight variation in the collagen deposition at the wound area between the treated and the control groups though the difference was not statistically significant. Absolute quantification of collagen fibers and/or biochemical estimation of intermediate metabolites of collagen biosynthesis may be required to reveal the marked difference between the treated and the control group. The mediating factors which influence the cellular changes as observed in this study were not considered.

In conclusion, this study provide the scientific prove that *V.amygdalina* leaves juice which is commonly used traditionally for the treatment of wound, has the capacity to accelerate wound healing through its effect on the wound tissue cellular activities. These have been linked to its phytochemical constituents. Further studies are required to isolate and characterize the active ingredients and elucidate their mechanisms of action.

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Photostress Recovery Time Among Commercial Drivers In Nigeria

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Abstract

Photostress recovery time (PSRT) test is a simple technique used to assess the macular function. Prolonged recovery time is attributed to the delay in regeneration of visual pigments after being bleached with a bright light. In car drivers with macular degeneration, photostress effects from opposing cars may bleach retinal pigments and cause a dramatic drop in visual acuity.

The study aims to determine the prevalence of abnormal PSRT and the association of PSRT with age, visual function and driving safety among commercial drivers in Nigeria.A cross-sectional descriptive study was carried out in Ilorin metropolis, a city in north central Nigeria. Of the estimated 4,729 registered commercial drivers, 327were selected using multistage sampling methods. Structured questionnaire was administered by trained research assistants. PSRT test and visual function tests were carried out by the principal researcher and an assistant.

Subject with normal healthy macular function should be able to read the visual acuity line just above the pre-exposure visual acuity line in 50-60 seconds. Patients with a macular problem may have recovery times lasting 1.5 to 3 min or longer. The study revealed a statistically significant association between PSRT and visual acuity as well as color vision ($p<0.05$). No association existed between PSRT and age, visual field and driving safety. Majority of the respondents with normal PSRT had fair driving safety.

Regular visual screening especially during license renewals may be the most useful tool for the detection of impaired visual function and delayed photo stress recovery time among commercial drivers.

Keywords: Photostress, Macular degeneration, Commercial drivers

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Introduction

Photostress is a psychophysical process which persists after light exposure. This is because dark adaptation takes time to restore visual sensitivity to its pre-exposure level.[1] The physiologic basis for retinal photostress response is believed to be a transient state of visual insensitivity caused by bleaching of the visual pigments of the retina when exposed to intense light.[2] Return of sensitivity is dependent on re-synthesis of the visual pigments, and re-synthesis requires adequate perfusion of the photoreceptors and the retinal pigment epithelium.[3] This further depends on the integrity of the choroids especially the Bruch's membrane and relatively independent of optic nerve integrity.

Delay in photostress recovery may pose serious danger to a driver when faced by a bright headlight of an oncoming vehicle. Photostress induces an afterimage, which is in the form of a scotoma.[3] Thescotoma subsequently recedes after which normal vision is restored. The PSRT test is used mainly to estimate the functional capability and the performance reserve of the photoreceptive elements of the macular.[4] It is a simple technique used to differentiate the cause of a reduced visual acuity (VA) in an eye between a lesion in the optic nerve and a macular disease.[5] Prolonged recovery time is attributed to the delay in the regeneration of visual pigments after being bleached with a bright light.[4] If the cause of the reduced VA is optic nerve, the bleaching of the retina will have no effect on the recovery time. Apart from being used as a diagnostic tool, this test has been used to monitor the progress of disease states such as chloroquinemaculopathy, nyctalopia on vitamin A therapy, age-related macular degeneration and as a method of determining their prognosis .[6]It has also been used to monitor the progression of maculopathy in high myopia. Horiguchi*et al*., found a significant delay of the recovery time in high myopia.[7]A prolonged PSRT or delayed adaptation was reported in glaucoma which mainly affects the ganglion cells. This suggests that a ganglion cell abnormality may delay recovery or that glaucoma may cause visual pigment abnormality.[7]The pathophysiology has however, not been established.[8]

Photostress recovery time (PSRT) varies with age. There is a difference in the recovery time for individuals over 40 years of age and younger patients. The acceptable recovery times of 50 to 60 seconds are more in line with individuals over 40 years of age. Recovery time for young healthy individuals with no

macular problem can be markedly less. Individuals with macular problem may have recovery time lasting 1.5 to 3 minutes or longer.[9]

Specifically, PSRT is a measure of the time it takes for an individual to read the line just above its pretest best acuity line backwards after adequate bleaching of the photoreceptors for 10 seconds. A variety of photostress tests have been advocated and assessed including pen torches (pen light), ophthalmoscopes, Maxwellian view optical systems, pupilometers, scanning laser ophthalmoscopes, and visually evoked potentials as light source.[10,11,12] A major criticism of PSRT testing has been the lack of a standardised technique in conducting the test.[3-5]This study describes the use of alkaline powered penlight to measure objectively the recovery of central vision after photostress. The use of penlight for the PSRT test was described by Glaser[1], with the primary goal of determining the efficacy of this simple test in determining optic nerve defects from macular disease in individuals with otherwise unexplained loss of central vision.[1] The technique was kept simple so that direct clinical application would be possible without cumbersome or expensive equipment. The result obtained in Glaser's study corresponded with that of Severin et al., (1987) who used an elaborate high intensity light flash system.[13]This study however, adopted Glaser's method of PSRT testing.

Materials and Methods

This cross-sectional descriptive study was carried out in Ilorin, the capital city of Kwara State, in the north central geographical zone of Nigeria. Of the estimated 4,729 commercial drivers who are registered with National Union of Road Transport Workers (NURTW), Road Transport Employers Association of Nigeria (RTEAN), State government owned transport service (Kwara Express) and other private owned motor parks in Ilorin metropolis, 327 consenting drivers participated in the study. The inclusion criteria for respondents in this study were drivers who are Nigerians by birth, who drive four wheel vehicles and convey passengers both intra and intercity, and can read the Snellen chart or understands the illiterate “E” very well. The approval for the study was obtained from the department of Epidemiology and Community Health, University of Ilorin.Informed consent was obtained from each respondent after detailed information about the study was read to them. The experimental procedures adhered to the tenets of the Declaration of Helsinki.

Participants' information and data were obtained by the use of a semi-structured interviewer administered questionnaires in each of the selected motor parks. The questionnaire elicited questions to obtain information concerning respondents' socio-demographical data, driving safety, photostress recovery time and other visual function assessment.

Visual function tests were done by the principal researcher and an assistant at designated spaces at the motor parks. The tests included distance VA testing done using the Snellen's chart and Illiterate 'E' chart for those who can read and those who cannot read respectively. The charts were placed 6 metres from the respondents and each eye was tested separately. Photostress test was done using battery energizer (AA 1.5V) pen torch with halogen bulb and a pocket electronic stop watch. The test was carried out on each respondent based on a similar procedure. The test light intensity was checked with luxmetre to ensure standardization. At 2cm, the penlight produced an illuminanceof 2,340 lumens/m^2. The batteries of the penlight were changed after being used for 15 respondents (after 150seconds). Reproducibility of PSRT value was tested in 15 respondents and there was no significant difference noted ($p>0.05$). The VA for each eye was tested first and then photostressed for ten seconds by looking at a penlight held 2 to 3 cm from the eye. The time required to read three letters on the Snellen test lines just larger than the best acuity was used as the end point.Color vision was tested using the Ishihara pseudoisochromatic plates. Visual field was tested by confrontation method using a good contrast test target. External eye examination using a pen torch and an internal eye examination using a direct ophthalmoscope was done. All findings were documented on clinical report sheet at the back of each respondent's questionnaire copy. A developed standard operating procedure (SOP) was strictly adhered to in the course of this study.

Data analysis was done using SPSS version 18.0. Appropriate test of significance was used to test statistics and the level of significance was predetermined at p-value of equal to or less than 0.05 at 95% confidence level.

Results

The age of the respondents ranged from 20 to 70years with a mean age of 46 $\pm$ 10.13 years. Majority (63.7%) of them were in the age group 40 – 59 years.
The respondents were all male. Many of the respondents (44.3%) had primary education while 17.1% had no formal education. Almost three quarter (73.2%), of the respondents had no other occupation other than driving(Table 1). Majority (93.6%) of the respondents were Yoruba (Figure 1).

About one-fifth of the respondents 64 (19.5%) had been involved in RTC in the past 5 years. Only 1 driver has had RTC thrice in the last 5 years. More than half (62.5%) of the road traffic crashes were without any injury sustained. The cause of the RTC was mainly vehicular factor 49 (76.6%), Table 2.

Likert scale was used to score the respondents to determine their driving safety. A few 48 (14.7%) of the respondents had good driving safety. Majority 231 (70.3%) of the respondents were found to have fair driving safety (Figure 2). Majority of the respondents 299 (91.4%) and 305 (93.3%) had normal PSRT in the right eye and left eye respectively. The mean PSRT was 22.45± 20.07 seconds and 18.27± 18.13 seconds for the right and left eye respectively (Figure 3). The scatter diagram in figure 4 revealed a non-significant increase in PSRT with increasing age, p = 0.435 and 0.634 in the right eye and left eye respectively.

Majority of the respondents had normal visual function as well as normal PSRT. Test of significance revealed a significant relationship between PSRT versus visual acuity and PSRT versus color vision respectively (P< 0.05). No significant association with visual field (Table 3).

Table 1: Socio-demographic Variables (N=327)

Age groups	Frequency	Percentage
20 – 29	15	4.6
30 – 39	70	21.3
40 – 49	94	28.7
50 – 59	114	35.0
60 and above	34	10.4
Educational Level		
None	56	17.1
Primary	145	44.3
Secondary	72	22.0
Tertiary	20	6.1
Quaranic	32	9.8
Vocational Training	2	0.6
Other Occupations		
None	239	73.2
Artisans	46	14.0
Trading	18	5.5
Self employed	4	1.2
Civil Servant	5	1.5
Farmers	13	4.0
Others	2	0.6

Table 2: Road Traffic Crash History among Respondents

Variables	Frequency	Percentage
Involvement in Road Traffic Crash in the Past (N=328)		
Yes	64	19.6
No	263	80.4
Number of Road Traffic Crash in the Past 5 Years (n=64)		
None	32	50.0
Once	25	39.1
Twice	6	9.4
Thrice	1	1.5
Seriousness of the Crash (n=64)		
No injury	40	62.5
Mild to serious injury	14	21.9
Fatal injury	10	15.6
Cause of crash (n=64)		
Road factor	4	6.3
Vehicular factor	49	76.6
Human factor	8	12.5
Animal	3	4.6

Table 3: Relationship between PSRT and Visual function

Visual function	PSRT Right Eye Normal (%)	Abnormal (%)	PSRT Left Eye Normal (%)	Abnormal (%)
Visual Acuity of Better Eye				
Adequate	276 (92.9)	21 (7.1)	283 (95.0)	15 (5.0)
Inadequate	23 (79.3)	6 (20.7)	23 (79.3)	6 (20.7)
	χ^2 = 6.451.	P-value = 0.011	χ^2 = 10.779.	P-value = 0.001
Visual Acuity of Second Eye				
Adequate	282 (93.7)	19 (6.3)	288 (95.7)	13 (4.3)
Inadequate	17 (68.0)	8 (32.0)	18 (69.2)	8 (30.8)
	χ^2 = 20.051.	P-value = 0.000	χ^2 = 27.862.	P-value = 0.000
Colour Vision				
Normal	275 (93.2)	20 (6.8)	282 (95.3)	14 (4.7)
Abnormal	24 (77.4)	7 (22.6)	24 (77.4)	7 (22.6)
	χ^2 = 9.220.	P-value = 0.002	χ^2 = 14.879.	P-value = 0.000
Visual Field				
Normal	244 (92.4)	20 (7.6)	240 (92.3)	20 (7.7)
Abnormal	55 (88.7)	7 (11.3)	59 (89.4)	7 (10.6)
	χ^2 = 0.912.	P-value = 0.340	χ^2 = 0.588.	P-value = 0.443

Figure 5 showed that majority of the respondents with normal PSRT had fair driving safety. Only a few (about 10%) had poor driving safety and the test of significant revealed a negative relationship between PSRT and driving safety ($p > 0.05$ in both eyes).

Discussion

There appears to be a wide variation in the average photostress recovery time among different studies; 10-50 seconds[3],27 ± 11seconds[1], 41.97 ± 17.34seconds[26], 5.95 ± 2.03seconds[18], 15seconds[14], 135.8 ± 63.9 seconds[15]. These variations could be

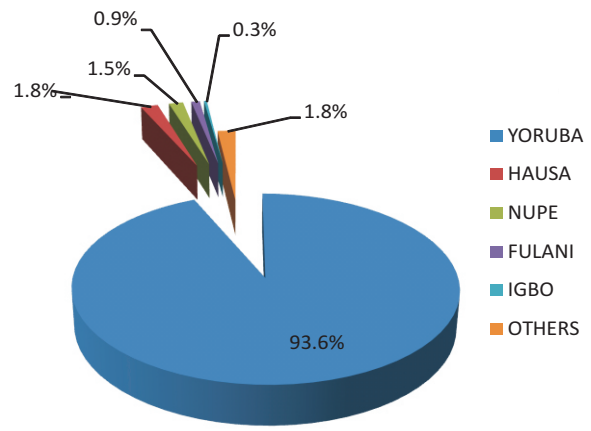

Figure 1: Ethnicity of the Respondents (N=327)

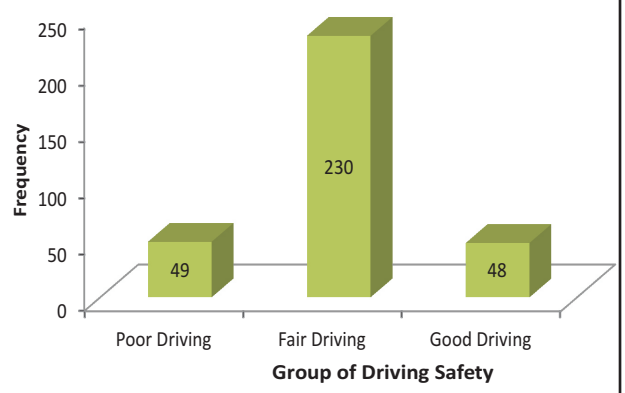

Figure 2: Outcome of Respondents' Driving Safety

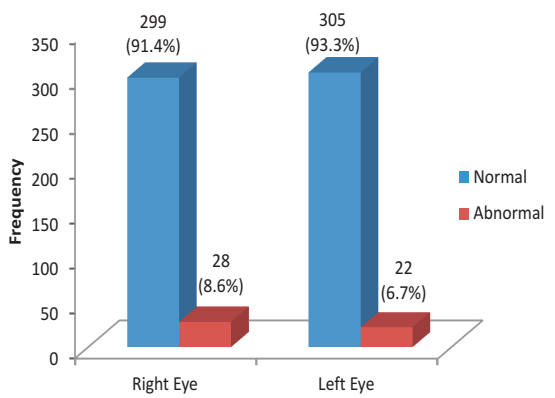

Figure 3: Outcome of Respondents' Photostress Recovery Time (N=327)

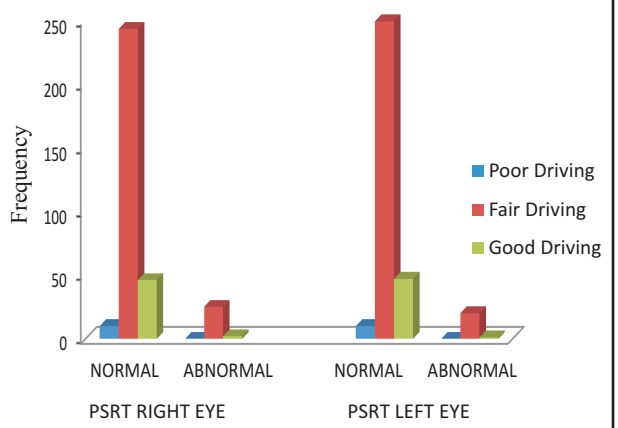

Figure 5: Relationship between PSRT and Driving Safety

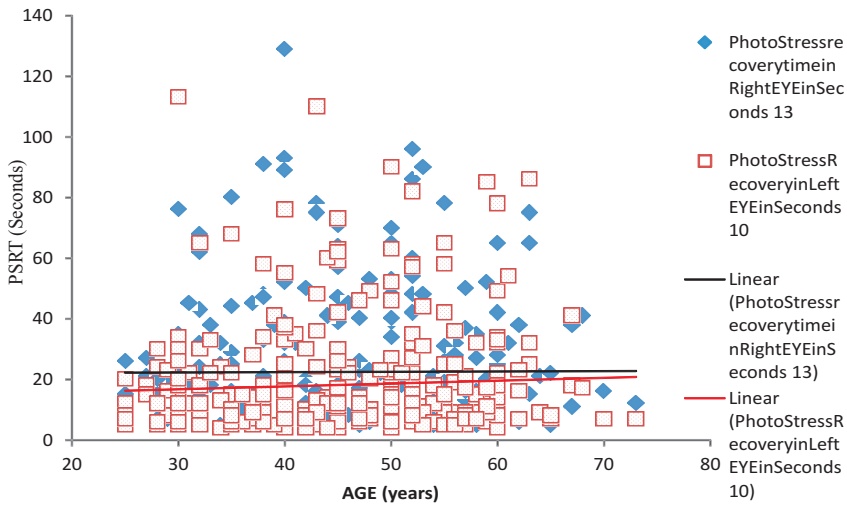

Figure 4: Relationship between PSRT and Age of respondents

attributable to non-uniform light intensity employed by the different studies for dazzling.[1, 3, 10-15, 18, 26]The mean PSRT for this study was 22.45± 20.07 seconds. This study adopted the method by Glaser et al.,[1] which is the use of pentorch. The choice of this method was because it was more readily available and easy to use. The average PSRT in our study was similar to that found in previous studies using similar short exposure flash like Ophthalmoscope, MAP, Eger macular stressometer(EMS) which tend to be more sophisticated.[3, 13, 14]The mean PSRT is generally lower than that obtained with other technique, [10–12, 15] which suggests the device does not bleach sufficient photopigment to challenge outer retinal physiology. The amount of photopigment bleached is dependent on the intensity and duration of the flash.[16] In addition; pupil size and eccentricity of viewing will affect the result. Elderly patients tend to have smaller pupils and majority (35%) of the respondents in this study was in this category. Therefore may need longer exposure to the light source for the photoreceptors to be fully bleached. Bleaching the photoreceptors with a direct ophthalmoscope for 30 seconds has been suggested to be the most reliable test of photostress as more photopigment is bleached and the effect of pupil size is limited.[4] As such, redesign of the test with a longer flash duration of greater visual angle may be appropriate.

PSRT in relation to Age

Photostress recovery time increases with photostress source luminance.[7, 17] It also increases with ageing, although different rates of increase are reported for different testing methods.[4, 17] Macular degeneration and other retinal disorders markedly prolong recovery times.[18]

The PSRT of 50 healthy subjects were measured in a study, using three clinically applicable techniques and a reference technique designed to bleach a consistent amount of photopigment and their ages ranged from 21 to 69. The agreement between each clinical technique and the reference technique was determined. Mean photostress recovery time differed for each of the techniques studied. Analysis of the data obtained with the reference and the best clinical technique showed that age was the only factor that had a significant effect on PSRT.[4]Increasing participant age was shown to result in a lengthening of the recovery time constant, of a magnitude comparable to previously published psychophysical data.[19]

This study revealed non-significant increase in the association between PSRT and increasing age (Figure 4). The revelation is contrary to the findings of a study done in Edo state, Nigeria.[20] The difference in the two reports despite applying the same method may be due to the marginal difference in the mean age of the participants in both studies (mean age: 46±10.13 and 33±12.74 for this study and the Edo state study respectively). Majority, 148 (45.5%) of the participants in this study were above 50 years of age and therefore more at risk of aging retinal degenerations that can affect PSRT when compared to the other study with a wide age range between 11 – 70years.[20]The increase in PSRT between age groups of 51-60 years and 61-70years and the decrease in PSRT between the age groups of 11-20 years to 21-30 years could be responsible for the positive relationship between age and PSRT as found in that study. It has previously been shown that PSRT is adversely affected by conditions affecting the macula, including central serous retinopathy,[7] age-related macular degeneration,[21] and diabetic maculopathy.[21–23] Disruption of the retinal pigment epithelium–retina relationship, because of serous retinal detachment or macular edema for example, has been shown to be an important factor in the prolongation of PSRT in such macular disease.[24] Importantly, PSRT deficits have been observed in asymptomatic subjects where visual acuity is relatively preserved, indicating that a suitably designed test might provide an effective indicator of early disease or disease progression.[25]

PSRT in relation to Visual function

The PSRT has been widely reported as a helpful screening clinical tool. PSRT has been proven by previous studies as a strong tool in determining the cause of reduced visual function as a problem in the macular or in the optic nerve. If the vision loss is owing to macular disease, the PSRT will be too long in contrast to when the cause of vision loss is attributed to optic nerve. This study revealed a positive relationship between PSRT and visual acuity as well as color vision. The relationship with visual field is not significant. Visual acuity is one of the best tests of macular function. If the macula is diseased, acuity falls. Color vision testing is also a good macular test because color is sensed in the cones which reside in the macular. This study therefore revealed an association with visual acuity and color vision because most of the respondents are above 50years of age and are therefore more predisposed to macula disorders. Some of the respondents on examination were also found to have some disorders that can affect macular function.

In a study to test the PSRT in 30 eyes from 15 patients with chronic open angle glaucoma, and 30 eyes from 15 individuals of a similar age group with no eye disorder, no association was found between visual acuity and recovery time for either group.[26] There was a relatively small significant association between age and recovery time in the control population and none in the glaucoma group. The reason for no significant association between PSRT and visual acuity could be attributed to the fact that glaucoma is a disease of the optic nerve head and optic nerve lesion does not affect

the PSRT.[2,3] Likewise the control group was without any visual disorder which could affect PSRT except for age which revealed a little association.

In a study to distinguish optic nerve conduction defects from macular disease in patients with otherwise unexplained loss of central vision, prolonged PSRT was found in 63 eyes with maculopathy and was not in 20 patients who had optic nerve disease.[1]

In a similar study which adopted the use of an eger macular stressometer (EMS), a different instrument from past studies, to determine association between PSRT with other measures of visual function. There was no evidence from results that EMS PSRT is affected by age related macular degeneration (AMD), contrary to this study report and previous reports using other photostress techniques.[1, 27, 28]Those patients with a central visual field defect or distortion had a similar EMS photostress recovery time as those with an intact central visual field in exudative (p = 0.56), atrophic (p = 0.38), or mixed AMD (p = 0.10). However the average EMS recovery time was similar to that found in previous studies using similar short exposure flash in healthy patients.[29]

In another study by Antonio et al., PSRT was found to be delayed by age. Cataract and primary open angle glaucoma groups did not affect the PSRT significantly.[30]There was no report on PSRT association with visual function in the previous study in Nigeria to compare this finding. The previous study[20] was carried out on healthy adult Nigerians (ie. without any visual disorder that can affect PSRT) with the aim of determining the influence of age on PSRT.

PSRT in relation to Drivingsafety

The test of the correlation between PSRT and driving safety(involvement in road traffic crash) is a test of the sensitivity of the drivers to glaring light sources such as a setting sun or the headlights of approaching cars. Subjects with increased glare sensitivity (i.e. tendency for delayed PSRT) may be more easily blinded in such conditions. Studies on accident statistics of subjects with increased glare sensitivity revealed high relative road traffic crash (RTC) risk values.[31]

In an on-road simulation study, Theeuwes*et al*., [32]found that headlight glare caused decreased recognition of objects along the roads, especially by elderly drivers. Skaar*et al*. [33] found that increased glare sensitivity correlated with decreased visual attention especially in elderly drivers. According to Higgins, Wood and Tait, onset of senile cataract, leading only to a mild decrease in visual acuity and selectively affecting glare appears much more detrimental for driving safety than decreased visual acuity produced by optical blur.[34]

Several visual functions including visual acuity, visual field, contrast sensitivity and glare recovery can be distinguished. These functions are basically independent of each other although many disorders and diseases of the eye cause impairments in more than one function. For example: cataract leads to decreased visual acuity but also to decrease contrast sensitivity and glare recovery (delayed PSRT). Loss of these visual functions is related to the presence of eye disorders and is best described in terms of visual disability such as loss of night driving ability.

This study revealed no significant association between PSRT and driving safety [p= 0.307 right eye and 0.266 left eye]. This could be explained by the fact that most people drive considerably fewer miles during the hours of darkness.[35] Another study revealed that more than half of RTC fatalities occur after dark.[36] This finding may be explained in part by changes in driver behavior associated with nightfall, such as increased alcohol consumption, increased driver fatigue and increased driving speeds due to lower traffic density. However, a number of authors have found an association between reduced illumination and driver safety that may contribute to reduced driving safety.[37]

In low illumination, physiological changes occur within the visual system resulting in degradation of both spatial and temporal resolution, a reduction in contrast sensitivity and poorer color discrimination. These changes are somewhat moderated during night driving by the presence of headlights, street lighting and reflective road markings, but visual processing still remains impaired compared with daytime illumination.[38] Drivers are generally unaware of the visual limitations of driving at night as this extrinsic lighting permits them to continue to see road signs and steer their vehicles effectively.[39] However, they will have more difficulty in detecting the low contrast and poorly illuminated objects due to delayed PSRT, and as such exhibit longer reaction times, resulting in longer stopping distances.[36,40]

The visual standards for licensure are based solely on performance in photopic conditions, although photopic visual acuity has been shown to be a poor predictor of the ability to recognize road signs at night.[41] Studies have also shown that the disparity between daytime and nighttime visual acuity levels increases with advancing age, with daytime levels becoming increasingly worse predictors of vision at night.[42] Low-contrast acuity in mesopic conditions, in the presence and absence of glare, is only tested in Germany prior to private or commercial driver licensing.[37]

Conclusion

The photostress test has been limited by a lack of standardization. There is tremendous variability in PSRT going by the available findings.

Having discussed the relationship between PSRT and driving safety as well as various visual functions, and having discussed the problems of the cut-off values, the next question is whether drivers should be periodically screened for impairments of vision and, if so, at what intervals. In the European Council Directive 91/439/EEC, there are no guidelines regarding periodic testing of drivers. In most member states, however, some form of visual evaluation takes place upon driving-licence renewal. An individual with progressive eye condition such as cataracts, glaucoma, etc., may be asked to provide vision reports from a specialist before license renewal. There may be need to issue a restricted license depending on the outcome of vision report in relation to recommended visual requirements for driving in Nigeria.

Positive associations between PSRT in relation to visual acuity and color vision were found in this study. If redesign and further development of the PSRT test could produce associations with driving safety especially at night, as well as with the severity and progression of macular degeneration, it may be an attractive test in clinical practice.

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Levels of Micronutrients and Heavy Metals in Cord Blood and Maternal Blood of Electric Stove Users Compared With Firewood Users.

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Abstract

Exposure to smoke from biomass fuel causes chronic diseases, but the mechanism underlying negative effects of household air pollution (HAP) is not exactly understood. The basis of the impact of HAP on maternal and fetal health was assessed by determining the levels of teratogenic heavy metals [Lead (Pb), Mercury (Hg)] and micronutrients associated with DNA methylation [Zinc (Zn), Iodine (I), vitamins B6 and B12, folic acid and homocysteine] in cord blood of babies and maternal blood of electric stove users compared with firewood users. Levels of Pb, Hg and Zn were determined by atomic absorption spectrophotometry (AAS). Folic acid, homocysteine, vitamins B6 and B12 were measured using enzyme link immunosorbent assay (ELISA).

Zn level was significantly higher whereas Pb level was significantly lower in sera of mothers using electric stoves compared with mothers using firewood. There were no significant differences in the levels of micronutrients and heavy metals in cord sera of babies born by mothers using electric stoves compared with the levels of micronutrients and heavy metals in cord sera from cord sera of babies born by mothers using firewood. Pb in maternal sera showed significant positive correlation with Pb in cord sera of babies born by mothers using firewood whereas vitamin B12 in maternal sera showed significant positive correlation with vitamin B12 in cord sera of babies born by mothers using electric stove.

Significantly high Pb level coupled with low Zn level in mothers using firewood might be responsible for adverse effects of firewood on the users. Our study therefore recommends Zn supplementation for those exposed to HAP from firewood. Thus, global DNA methylation is proposed as further study to explain the effects of HAP.

Key words: Indoor Pollutants, DNA Methylation, Mothers, Neonates, Micronutrients supplementation.

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Introduction

Indoor air pollution can be traced to prehistoric times when human first used fire for cooking, warmth and light[1]. Approximately half of the world's population and up to 90% of rural households in developing countries rely on unprocessed biomass fuels in the form of fire-wood for domestic uses[2]. Soot from wood combustion in poorly ventilated places produce high levels of indoor pollution[3, 4] containing sulfur oxides, nitrogen oxides, carbon monoxide and potentially carcinogenic compounds (polycyclic aromatic hydrocarbons PHAs, CO_2, NO, benzene, formaldehyde, dioxins and free radicals)[5] which are known to cause cancers, cataracts, pulmonary diseases[6], increased risk of stillbirth, low birth weight and impaired cognitive development[7, 8]. Modernization and improved socio-economic status have been accompanied by a shift from biomass fuels such as wood to petroleum products (such as kerosene and gas) and electric stove[9, 10]. However, most households in Nigeria still use firewood because of its relative cheapness and availability. Other households combine the uses of kerosene stoves with firewood, gas stoves and electric stoves depending on economic situations[11]. Mothers and babies during cooking are mostly exposed to fine particulate matters from indoor air pollutants[9].

Mechanism by which the constituents of HAP initiates or propagates the harmful effects is not fully understood but one of our previous studies suggested induction of oxidative stress and inflammation[4]. Some groups have also shown that both oxidative stress and nitric oxide can affect DNA methylation status[31]. Nutrition is known to play an important role in DNA methylation, as many dietary micronutrients such as folate, vit. B12 and Zn are directly involved in DNA methylation pathways though some of these micronutrients are antagonized by heavy metals such as Pb and Hg[32]. Micronutrients are also important in determining genomic stability as it affects all relevant developmental pathways such as activation/ detoxification of chemicals preventing DNA oxidation, DNA repair, apoptosis and DNA synthesis[33]. Folate and vitamin B12 deficiencies cause DNA hypomethylation, a defect that leads to chromosome loss[33].

Previous studies have reported increased levels of heavy metals such as Pb and Cd in animal products processed with firewood[12] and analysis of ashes from burnt firewood revealed significantly high Pb level[12].

Heavy metals have been implicated to be antagonistic to the availability and functions of essential micronutrients[32]. Thus, it is likely that chronic exposure to components of HAP will affect micronutrients associated with DNA methylation in the users of fire-wood. This hypothesis is reinforced by the fact that HAP from firewood contains heavy metals, induces oxidative stress, regulates DNA methylation and increases susceptibility to chronic diseases. The outcome of this study will predict future effects of firewood smoke on babies born to the users, thus may lead to recommendation of cleaner mode of cooking or recommend use of supplements that contains micronutrients which modulates DNA methylation to those that must use biomass fuels.

Despite significant negative health effects of exposure to HAP, less information is available to explain these observations. The study therefore determined micronutrients that modulate DNA methylation (Zn, I, vit B6, vit B12, folate and homocysteine) and some heavy metals (Pb and Hg) in cord blood of babies and mothers using firewood compared those using electric stoves.

Materials and method

Study Site and Subjects

A cross sectional study was carried out to determine the micronutrient and heavy metal status of pregnant mothers and cord blood of babies born by women who engaged in the use of fire-wood or electric stoves for cooking. The subjects were selected from Moniya Maternity Centre and University College Hospital, Ibadan, Nigeria. Out of 229 women recruited, only six of them cooked solely with firewood and six cooked solely with electric stove for at least 10years were included. Questionnaire was administered to determine dietary pattern to exclude those with health defects that could impair micronutrient status and those on any supplement or compulsory medication. Also excluded were those with pregnancy complications, with any infectious micro-organisms and those using other cooking methods apart from fire-wood and electric stoves. Ethical consideration to carry out the study was obtained from Oyo State Ministry of Health, Nigeria. All the pregnant mothers consented to participation in the study.

Blood, urine and faecal samples were collected by trained personnel and were processed as previously described to exclude those with any infectious agent[13, 14]. Serum levels of Pb, Hg and Zn were determined using Atomic absorption spectrophotometry (AAS). Homocysteine, vitamins B6 and B12 were measured using Enzyme link immunosorbent assay (ELISA) as previously described[15]. The quantification of iodine was performed by a potentiometric method using an Iodide ISE[16].

Results

Zn level was significantly higher whereas Pb level was significantly lower in sera of mothers using electric stoves compared with mothers using firewood (Table 1). There were no significant differences in the levels of micronutrients and heavy metals in cord sera of babies born by mothers using electric stoves compared with the levels of micronutrients and heavy metals in cord sera of babies born by mothers using firewood (Table 2). The correlations between levels of micronutrients or heavy metals in maternal sera compared with levels of micronutrients or heavy metals in cord sera were presented in Table 3 and 4. Pb in maternal sera showed significant positive correlation with Pb in cord sera of babies born by mothers using firewood (Table 4) whereas vitamin B12 in maternal sera showed significant positive correlation with vitamin B12 in cord sera of babies born by mothers using electric stove users (Table 3).

Table 1: Mean serum levels of micronutrients and heavy metals in women using electric stoves compared with those using firewood.

Parameters	Electric stoves (n=6)	Firewood (n=6)	p-value
Zn (µg/dl)	99.09±5.3	81.18±6.2	0.019*
Pb (µg/dl)	3.04±0.5	6.41±1.0	0.046*
Hg (µg/L)	9.17±0.9	9.23±0.5	0.917
I (µg/L)	54.45±6.9	49.88±10.4	0.561
Vit B6 (pg/mol)	6.98±1.45	7.19±0.5	0.825
Vit B12 (pg/mol)	441.49±72.0	401.01±42.1	0.448
Folic acid (ng/ml)	9.55±2.7	9.96±0.6	0.807
Homocysteine (µmol/L)	7.78±1.6	7.68±0.5	0.924

*Showed significant differences

Table 2: Mean cord serum levels of micronutrients and heavy metals in women using electric stoves compared with those using firewood.

Parameters	Electric stoves	Firewood	p-value
Zn (µg/dl)	124.70±12.3	121.84±14.4	0.807
Pb (µg/dl)	4.15±1.3	4.29±1.4	0.900
Hg (µg/L)	10.57±0.5	11.43±2.3	0.565
I (µg/L)	64.76±3.3	69.95±14.4	0.576
Vit B6 (pg/ml)	8.37±1.7	7.49±1.7	0.559
Vit B12 (pg/ml)	372.37±25.2	322.66±43.5	0.162
Folic acid (ng/ml)	10.42±2.7	10.19±1.4	0.899
Homocysteine (µmol/L)	10.44±1.0	10.09±2.1	0.807

Table 3: Correlation of levels of micronutrients and heavy metals in cord sera with the levels in sera of mothers using electric stoves

		r-value	p-value
Electric	Zinc (Maternal) & Zinc (Cord blood)	-.828	.379
	Pb (Maternal) &Pb (Cord blood)	-.946	.211
	Hg (Maternal) & Hg (Cord blood)	.640	.558
	Iodine (Maternal) & Iodine (Cord blood)	.286	.815
	Vit B6 (Maternal &Vit B6 (Cord blood)	.759	.452
	Vit B12 (Maternal) &Vit B12 (Cord blood)	1.000	.009*
	Folic Acid (Maternal) & Folic Acid (Cord blood)	.985	.112
	Homocysteine (Maternal) &Homocysteine (Cord blood)	.965	.168

*Showed significant difference

Table 4: Correlation of levels of micronutrients and heavy metals in cord sera with the levels in sera of mothers using firewood.

		r-value	p-value
Firewood	Zinc (Maternal) & Zinc (Cord blood)	.091	.942
	Pb (Maternal) &Pb (Cord blood)	1.000	.006*
	Hg (Maternal) & Hg (Cord blood)	-.985	.109
	Iodine (Maternal) & Iodine (Cord blood)	.122	.922
	Vit B6 (Maternal &Vit B6 (Cord blood)	.979	.132
	Vit B12 (Maternal) &Vit B12 (Cord blood)	.693	.513
	Folic Acid (Maternal) & Folic Acid (Cord blood)	.664	.538
	Homocysteine (Maternal) &Homocysteine (Cord blood)	.985	.110

*Showed significant difference

Discussion

Exposure to HAP from biomass smoke has been implicated in inflammation and in the development of respiratory diseases[17, 18]. Also, studies have linked significant HAP exposure of pregnant women with increased adverse effects in foetus[19]. Although the underlying mechanisms for HAP-related injury are still being investigated, exposure induced-oxidative damage has been alleged to play an important role[4, 20].

In the present study, mean serum level of Pb in pregnant women using firewood was significantly higher compared with mothers using electric stove. This is an indication that Pb from firewood smoke might have entered the blood circulation of women using firewood. A previous study reported accumulation of Pb in firewood smoke[12, 22]. The present study also observed significant positive correlation of Pb in maternal and cord blood of firewood users. Thus, supporting the fact that Pb in the bloodstream of mothers might have been transferred to the fetus. Our result corroborates a previous finding that Pb readily crosses the placenta by passive diffusion and has been detected in the fetal brain as early as the end of the first trimester[23]. Elevated Pb levels in pregnancy have been associated with gestational hypertension, spontaneous abortion, low birth weight, and impaired neurodevelopment[24]. Epidemiological and toxicological studies showed that epigenetic alterations induced by Pb may be the key mechanisms linking Pb exposure to its toxicity[25, 26]. This is in line with a report that Pb induces oxidative stress causing damage to membrane, DNA and proteins. Therefore, increased level of Pb as observed in the present study might induce epigenetic changes and oxidative stress, thus the reason for the adverse effects of Pb in firewood users.

Our present study showed significantly reduced Zn level coupled with raised level of Pb in mothers using firewood. Zn and Pb compete for similar binding sites on the metallothionein-like transport protein in the gastrointestinal tract. Excessive inhalation or indirect ingestion of Pb from firewood smoke might have caused decreased absorption of Zn thus reducing blood Zn level in firewood users. Dietary supplementation with Zn and in combination with ascorbic acid and thiamine was shown to reduce Pb toxicity in humans[26]. Thus, the protective effects of zinc against oxidative damage may support its recommendation for mothers who must use firewood.

There are data suggesting Pb-induced alteration in gene expression by interacting with zinc-binding sites on DNA-associated protein (protamine).

Zn has a relationship with many enzymes and prevents cell damage through activation of the antioxidant system[27]. Zn deficiency has been shown to increase lipid peroxidation in rat tissues and Zn has also been reported as an essential component of the antioxidant defense system[28]. Thus, reduced Zn level coupled with raised level of Pb in mothers using firewood further support oxidative stress hypothesis as a basis of HAP-induced adverse effects in mothers that use firewood.

Cord blood analysis has been reported to provide individual measure of prenatal exposure to HAP. Prenatal exposure to pollutants in HAP has been shown to increase chances of neuro-developmental disorders[29], which may be modified by micronutrients. In this study, vitamin B12 in maternal blood showed significant positive correlation with cord blood vitamin B12 in electric stove users. This implies adequate transfer of vitamin B12 from mothers to fetuses in utero. A research showed that adequate intake of vitamin B12 and folate in pregnancy prevents neural tube defects such as spinal bifida, low cognition as well as megaloblastic anaemia in babies[30]. Therefore, women that use electric stove might have better pregnancy outcomes than firewood users. However, in the firewood users, the level of Pb in the mothers showed significant positive correlation with cord Pb level. This suggests potential adverse effects of Pb in the babies born to mothers exposed to HAP from firewood smoke.

Conclusion:

Significantly high Pb level coupled with low Zn level in mothers using firewood compared with mothers using electric stoves might be responsible for adverse effects of firewood on the users.

Limitation:

Low number of selected participants is due to strict adherence to selection criterion. Most women in Nigeria domestically use either kerosene stove, gas stove or combination of the two methods of cooking.

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Knowledge And Attitudes Of Pregnant Women Towards Anaesthetic Techniques For Caesarean Delivery In A North Central Teaching Hospital

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Abstract

As caesarean deliveries increase globally, more women will be exposed to anaesthesia as part of the surgical procedures. Evaluation of the knowledge and attitudes of parturient will guide patient education and counselling and may improve overall satisfaction with caesarean delivery.

This was a cross sectional study involving parturient aged 21-47 years scheduled for elective caesarean delivery at the University of Ilorin Teaching Hospital, Ilorin, Nigeria. Sampling method was purposive, informed consent was obtained from all participants and data collection was through an interviewer-administered questionnaire. Data analysis was by SPSS version 20.0 and $p<0.05$ was significant.

Awareness about anaesthetic techniques for caesarean delivery was 85(78%) but 31 (37%) were adequately informed about the techniques. The preferences of parturient for specific anaesthetic techniques favoured regional anaesthesia (spinal [68.3%], epidural [5%]), compared to general anaesthesia (18.3%). Educational level, previous anaesthetic experience, types of occupation, partner's level of education among others had likelihood of improving the knowledge and choice of anaesthetic techniques for caesarean delivery among the respondents.

Conclusion from the study revealed that spinal anaesthesia was preferred for caesarean delivery similar to the global trend due to its perceived safety. Adequate information at antenatal clinic and involvement of anaesthestists may help to improve knowledge and acceptance of anaesthetic techniques by parturient.

Keywords: Knowledge, Attitudes, Pregnant women, Anaesthetic techniques, Caesarean section.

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Introduction

Anaesthesia remains an important component of surgical procedures; its introduction was one of the milestones in surgical practice. At inception, caesarean delivery was associated with a high complication rate to both the mother and the baby. However, the associated risk has reduced significantly and the procedure is now safer due to improved surgical technique, better anaesthesia, effective antibiotics and safer blood transfusion.[1]

The choice of any anaesthetic method is determined by maternal health status, urgency of the surgical delivery as well as patients' desire and surgeon's preference.[2] In addition, safety of anaesthetic technique, degree of maternal comfort, the depressant effects of the agent to the foetus and the extent of provision of optimal working condition for the obstetrician also influence the choice of technique.[2] Regional anaesthesia (RA) in surgical specialties has gained popularity worldwide. Subarachnoid block, epidural, and combined spinal-epidural anaesthesia are now attractive options for caesarean deliveries. Regional anaesthesia is increasingly being preferred because it is considered safer, associated with reduced blood loss, eliminates the trauma of awareness under general anaesthesia[3] and allows immediate interaction between mothers and their newborns.[4] Reported drawbacks of RA include fear of backache, headache and inadequate anaesthesia.[5] In our environment, attitudes to anaesthetic techniques have been negatively affected by misinformation, myths and wrong cultural beliefs. Therefore, this study determined the knowledge and attitudes of pregnant women, scheduled for elective caesarean delivery towards anaesthetic techniques. This will provide opportunity to correct misconceptions on anaesthetic techniques for caesarean sections through adequate information of parturient.

Materials and methods

The study was a prospective cross sectional descriptive study conducted at the University of Ilorin Teaching Hospital, Ilorin following the institutional ethical review committee approval between May 2015 and February 2016. Participants were parturient scheduled for elective caesarean delivery irrespective of the indication. Parturient admitted for vaginal deliveries or other instrumental interventions were excluded from the study. Patient who refused to participate in the study were also excluded. The

sampling technique was purposive, involving inclusion of all consenting eligible parturient until the sample size was completed. Informed consent was obtained from all eligible participants following which an interviewer-administered questionnaire was used for data collection. All required pre-operative preparations were made for all participants including review by anaesthesiologist and blood grouping and cross matching.

The information obtained from participants included demographic factors, knowledge and attitudes of the parturient about anaesthetic techniques for caesarean delivery, the sources of the information, individual preferred choice for anaesthesia and the reasons for the choices. Provision was made for an interpreter in each of the local dialects who assisted in the interpretations of the questionnaire to women who could not communicate in English language. The recruited interpreters were non-health personnel who were educated and trained about meaning of terms to ensure uniformity of interpretation followed by pretest before commencement of data collection. The sample size was determined using Fisher's formular.[6]

Data collation and editing was done manually to detect omission and ensure uniform coding; and descriptive data analysis was done to obtain frequencies and percentages using the Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago Illinois.). Age of the parturient was expressed in mean with standard. Fisher's exact test was calculated between knowledge of parturient about options of anaesthetic technique and education level, previous exposure to anaesthesia, sources of information and occupation of the respondents. There was no conflict of interest in the conduct of the study.

Results

A total of 109 respondents participated in the study; the mean age was 30.8±5.3 (range 21-47) and median gravidity of 2 (range 0-7) while 60(76.9%) had tertiary level of education as shown in Table 1. Awareness about anaesthetic techniques for cesarean delivery was 85 (78%) while the commonest sources of information were the obstetrician (34.1%) and friends

Table 1: Biosocial characteristics of participants

Parameter	Frequency (%)
Age (years)	
Mean : 30.8±5.3	-
Range: 21-47	-
Parity	
Median: 2.0 (range 0-7)	-
Parturient level of education	
None	1(0.9)
Primary	2(1.8)
Secondary	15(13.8)
Tertiary	60(55.1)
Not stated	31(28.4)
Parturient occupation	
Artisan	6(5.5)
Housewife	10(9.2)
Civil servant	34(31.2)
Trading	50(45.9)
Not stated	9(8.3)
Partner's occupation	
Retiree	2(1.8)
Artisan	7(6.4)
Driving	3(2.8)
Trading	24(22.0)
Civil servant	64(58.7)
Not stated	9(8.3)
Partner's level of education	
None	4(3.7)
Primary	2(1.8)
Secondary	17(15.6)
Tertiary	77(70.6)
Not stated	9 (8.3)

Table 2: Knowledge about anaesthetic techniques for caesarean delivery

Parameters		Frequency (%)
Awareness about techniques :	Yes	85(78)
	No	24(22)
Adequate information about techniques:	Yes	31(36.5)
	No	54(63.5)
Sources of information		
Radio		1(1.2)
Films		2(2.4)
Obstetrician		29(34.1)
Nurses		1(1.2)
Anaesthetists		9(10.6)
Read about it		7(8.2)
Friends		27(31.8)
Family members		9(10.6)
Anaesthetic techniques known		85(100)
General anaesthesia		15(17.7)
Spinal		66(77.7)
Epidural		4(4.7)
Are you aware of any complication of the technique :	Yes	32(29.4)
	No	77(70.6)
Total parturient aware of complication		32(100)
Death from anaesthesia		20(62.5)
Severe hypotension		9(28.1)
Wrong endo-tracheal tube placement		2(6.3)
Meningitis		1(3.1)

Table 3: Previous experiences and current attitude to anaesthetic techniques

Parameters:		Frequency (%)
Have you undergone surgery before?	Yes	66(60.6)
	No	(10.1)
	No response	32(29.4)
Which anaesthetic technique was used (n=66)		
General anaesthesia		20(30.3)
Spinal		39(59.1)
Epidural		7(10.6)
Any problem from the previous anaesthesia?	Yes	14(21.2)
	No	52(78.8)
Description of experiences with the previous anaesthesia (n=109)		
Satisfied		104(95.4)
Not satisfied		3(2.8)
A terrible experience		2(1.8)
Concerns about anaesthetic techniques? (n=109)		
No concern at all		29(26.6)
Fear of delayed recovery or death Fear of inadequate anaesthesia		38(34.9)
Fear of inadequate anaesthesia		5(4.6)
Fear that something may going wrong		13(11.9)
Desire to see baby immediately after delivery		24(22.0)
Preferred anaesthetic techniques for this surgery (n=80)		
General		18(22.5)
Spinal		58(72.5)
Epidural		4(5)
Reasons for choosing anaesthetic technique (n=80)		
Spinal techniques:		
I have undergone it before		11(13.8)
I think it is safer		64(80)
Desire to see baby		5(6.3)
General anaesthesia:		
Unwillingness to see during surgery		2(40)
Previous experience		1(20)
The only offered choice		2(20)

Table 4: Univariate analysis for respondents' knowledge about anaesthetic techniques for caesarean delivery

Variables	Fisher's exact test	p-value	Significance
Educational level	10.422	0.004	s
Partners' educational level	10.597	0.014	s
Pre-delivery counseling	16.449	0.000	s
Occupation	18.513	0.000	s
Past exposure to anaesthesia	11.378	0.002	s

s-significant

with previous experience of anaesthesia (31.8%). Despite high knowledge rate about anaesthetic techniques for caesarean delivery, only 31 (37.2%) were adequately informed about the methods and showed evidence of complete understanding on the various anaesthetic techniques as they asked their healthcare providers no further questions up to the point of provision of the service, Table 2.

The majority of the respondents ([80], 73.4%) preferred spinal anaesthesia for caesarean section; and the reasons given were safety ([64], 80%), previous experience with spinal anaesthesia ([11], 13.8%), and desire to see their babies at delivery ([5], 6.3%). Among the 85 respondents who have heard about different anaesthetic techniques for caesarean delivery, 58 (68%), 15 (18%) and 4 (5%) expressed desires for spinal, general and epidural anaesthesia respectively, Table 2.

The remaining 8 (9.4%) parturient expressed no preference for any particular techniques despite prior counseling on the available anaesthetic methods. Despite strong popularity of spinal technique among the participants, few women still preferred general anaesthesia due to desire to avoid seeing things during surgery 2 (1.8%), previous experience 1 (0.9%), and because it was the only available choice 2 (1.8%).

Majority of the respondents, 77 of the 85 (90.1%), with prior knowledge of the anaesthetic methods for caesarean section knew about the techniques more than a year

earlier; sixty six (85.7%) of whom have had previous surgical operations under different forms of anaesthetic method, spinal 39 (59.1%), general 20 (30.3%) and epidural anaesthesia 7 (10.6%) respectively, Table 3.

Although, satisfaction with the previous anaesthetic techniques was admitted by 59 (89.4%) of the respondents, anaesthesia-related complications occurred in 14 (12.8%) of study group. Of the 24 (22%) respondents who lacked prior knowledge of the various anaesthetic techniques for caesarean delivery in this study, 20 (83.3%) expressed desirability for their surgery to be done under spinal anaesthesia while GA and epidural/combined spinal epidural were chosen by 2 (8.3%) respectively. Two respondents (8.3%) did not state any preferred technique following counseling on the available anaesthetic techniques for caesarean delivery, Table 3.

The observed factors that likely influenced the choice of anaesthetic methods for caesarean sections in our respondents are their educational level, pre-delivery anaesthesia counseling, exposure to anaesthesia in the past, occupation types and level of education attained by their partners, Table 4. More than 50% of the women with at least secondary education were aware and chose methods of anaesthesia for caesarean delivery compared with those with primary or no formal education who chose no anaesthetic technique for their deliveries (p value= 0.004); and 92% of respondents who had pre-delivery anaesthesia counseling during ante-natal care chose anaesthetic technique in comparison with only 62.5% that chose anaesthesia methods in those with no prior discussion about existence of various methods (p-value=0.000). Majority of the traders (92.6%) among the respondents were more aware about anaesthetic methods compared with 70.3% of the civil servants and the difference was statistically significant, (p value = 0.000). Parturient whose husbands were educated demonstrated statistically significant greater awareness of the various forms of anaesthetic methods for their caesarean deliveries compared with respondents whose husbands had no formal education, p value = 0.014, Table 4.

Discussion

In the last decades, the rate of caesarean delivery has been on the increase globally despite the recommendation of the World Health Organization (WHO) to keep it below 10-15%.[7] The experience in UITH Ilorin is not different from the global trend, an increase from 1 in 26 deliveries in 1990 to 1 in 5 deliveries in 1999,[8] with general anaesthesia and infiltration with local anaesthetic agent being the only anaesthetic methods utilized. In contrast, spinal anaesthesia has become the commonest anaesthetic technique for caesarean section in our institution in the last ten years but there has been no study to assess the knowledge and attitudes of pregnant women to all the available anaesthetic methods for caesarean deliveries. The high knowledge of the various anaesthetic techniques in this study is comparable to 82.4% reported among Pakistani women; although, earlier reports from third world countries showed much lower figures.[9, 10] The high knowledge rate among the respondents in this study could be due to the high literacy level (96.1%) of the respondents.

In this study, level of education of parturient, pre-delivery anaesthesia counseling, trade-based occupation and educated husband were identified as the likely factors responsible for the observed high knowledge rate. These are consisted with the findings of previous studies[3,11] that education and previous anaesthetic experience have strong tendency to increase the knowledge of women on the various anaesthetic techniques for caesarean section. In contrast to one of the findings in this study, however, some studies did not find previous exposure to anaesthesia as promoting factor of awareness of anaesthetic methods.[12,13] Interestingly, previous experience in terms of the technique and pre-delivery anaesthesia counseling was emphasized as the important factor that enhances knowledge.[11]

We also found that majority of the surveyed women preferred regional to general anaesthesia for their caesarean deliveries unlike the high preference of pregnant women for GA from the outcome of similar study from another developing country.[11] The reasons given for refusing regional anaesthesia by the subjects included fear of needle pain, inadequate anaesthesia, back pain and leg numbness. Fear of failure to recover from general anaesthesia was the major reason for refusing GA; other reasons for refusing GA were fear of side effects on the babies, post-operative throat pain and counsel of relatives. The high requests for spinal than epidural anaesthesia by the parturient was probably acceded to by the anaesthetists due to its shorter-time-to-establish and high success rate in comparison with epidural anaesthesia.

In the same study, anaesthetists were the major source of information about the anaesthetic techniques[5] whereas our study revealed that obstetricians and friends of the parturient (34.1% and 31.8% respectively) were the major sources of information about the various anaesthetic techniques.

In the United States, 1.6% of maternal deaths were anaesthesia-related; although the case fatality of general GA was reported to be falling with a slight increase in that of spinal anaesthesia; despite a risk ratio of 1.7 between general and spinal anaesthesia, the rate of spinal anaesthesia continues to rise.[4] The high preference for regional anaesthesia by respondents (73.3%) in this study lends credence to the general notion that regional anaesthesia is associated with low

mortality[4] and helps pregnant women achieve their desires of early interactions with their babies in the immediate post-delivery period.

Arguably, the previous experiences of parturient about anaesthetic techniques for caesarean sections can be indirectly evaluated by assessing the level of satisfaction of women with the procedure. Maternal satisfaction with previous anaesthesia in this study was 89.4% similar to a report of 81.4% maternal satisfaction following spinal anaesthesia for caesarean deliveries in Pakistan.[14] In Pakistan, satisfaction with spinal anaesthesia was 74.09% for intra-operative pain/discomfort and 76.83% for postoperative back pain with a 53.6% expressed desire to use spinal anaesthesia again.[2] Despite the expressed high maternal satisfaction following regional anaesthetic technique, a systematic review showed no evidence that supports superiority of RA over GA in terms of major maternal and newborn outcomes.[3]

Generally, anaesthetic technique for caesarean section can be expected to vary from one facility to another due to availability of resources and expertise but there is paucity of studies for reasons for refusal or acceptance of a particular type of technique of anaesthesia. The reasons for this seem to be multi-factorial including cultural differences, lack of knowledge of the various techniques and false beliefs.[15] Culture features prominently as the reason for low demand for RA in developing countries.[9,10]

This survey showed that fewer respondents (37.2%) had adequate information about the anaesthetic techniques for caesarean section despite high awareness. This may represent the knowledge of the general population on anaesthetic techniques. This may be due to very little or no involvement of anaesthetists in the pre-delivery anaesthesia counseling and education during ante-natal care. Counselling and education offered by trained anaesthetists have a greater likelihood to be more detailed and possibly easier to understand by the parturient. Therefore, it will be necessary to involve them in such prenatal education and counseling.

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Hypoglycaemia From Misuse Of Oral Hypoglycaemic Agent In Patients With Lower Urinary Tract Symptoms In Ilorin, Nigeria.

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Abstract:

Hypoglycaemia can be defined biochemically as level of blood glucose below 2.8 mmol/L (50mg/dL) in otherwise healthy adult. It is often characterized by variety of symptoms including confusion, loss of consciousness, seizures and rarely death may occur. The most common cause of hypoglycaemia is medications used to treat diabetes. Although, only some few recent studies found a significant association between factors in the metabolic syndrome and presence of benign prostatic hypertrophy (BPH); there has been no documented beneficial effect of the use of oral hypoglycaemic agent in the treatment of lower urinary tract symptoms (LUTS) from BPH either in diabetes or non-diabetes individuals.

The incidence of diabetes mellitus is high about the same decades of life when the prevalence of LUTS/BPH is also at its peak. This coincidence may influence the pattern of presentations of such patients with LUTS associated with BPH especially in the rural African settings where patients tend to share their medications or prescription because of similarity in perception of their symptomatology.

Case series of five patients with lower urinary tract symptoms, who had abused oral hypoglycaemic agents and presenting in various stages of altered consciousness and frank hypoglycaemic coma, were presented to illustrate this occasional occurrence and suggestion on how to militate this trend.

In conclusion, misuse of oral hypoglycaemic agents (OHA) to treat LUTS with subsequent presentation in hypoglycaemic coma should be of concern to the health care provider especially the urologist. It is of no benefit and constitutes drug abuse that should be discouraged.

Keywords: drug abuse, hypoglycaemia, lower urinary tract symptoms.

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Introduction

Blood glucose level of 2.8mmol/L is use to define the biochemical level of hypoglycaemia in healthy adult, however, in diabetic patient a much higher blood glucose level of 3.9mmol/L and below is used for biochemical diagnosis of hypoglycaemia[1,2]. Hypoglycaemia is often characterized, depending on how low the blood glucose level, by variety of symptoms including clumsiness, confusion, loss of consciousness, seizures and rarely death may occur. The most common cause of hypoglycaemia is medications used to treat hyperglycaemic state (diabetes mellitus)[1].

Although, several studies have examined possible association between diabetes and benign prostatic hyperplasia (BPH) only some few recent studies found a significant association between factors in the metabolic syndrome and presence of BPH[3]. However, there has been no documented beneficial effect of the use of oral hypoglycaemic agent in the treatment of lower urinary tract symptoms (LUTS) from BPH either in diabetes or non-diabetes individuals.

The incidence of diabetes mellitus has been reported to be common within a certain age ranges of the population, incidentally however, the prevalence of LUTS/BPH also peaked at about this age ranges[4]. This coincidence may influence the pattern of presentations of such patients with LUTS associated with BPH especially in the rural African settings where patients tend to share their medications or prescription because of similarity in perception of their symptomatology.

Case series of five patients, seen in our institution with lower urinary tract symptoms, who had abused oral hypoglycaemic agents and presenting in various stages of altered consciousness and frank hypoglycaemic coma, were presented to illustrate this unusual and very occasional presentation and suggestion on how to militate this trend.

Material and Methods

This was a retrospective review of a prospectively collected data of five patients that were managed between January 2008 and December 2015. All the patients presented with altered state of consciousness following ingestion of drugs that was given to them by either friends or family member to treat urinary symptoms. A diagnosis of drug-induced hypoglycaemia was confirmed biochemically in all of them and they all responded to glucose infusion

treatment. They were all non-diabetes following further evaluation and they were treated for the various causes of their individual LUTS.

Case series

Case 1

A 65-years-old businessman was admitted to the emergency unit of the hospital unconscious having ingested suspected oral hypoglycaemic agent the night before presentation. He has had preceding difficulty with voiding that was characterized by bothersome LUTS which has being progressively worsening over the previous two years. No urinary retention, no haematuria and no symptom referable to the upper urinary tract. He is not a known hypertensive or diabetes patient. He took some pills, that was later found to be Diabinese(R) (chlorpropamide), which was "prescribed" by a friend who had 'similar' symptoms; he was found unconscious some hours after the medication.

He was admitted with Glasgow Coma Score (GCS) of 8; he was not febrile, not pale, anicteric and he was well hydrated. The chest and cardiovascular system were normal. Abdominal examination revealed a full bladder that was relieved by urethral catheterization and an enlarged prostate with benign features on digital rectal examination.

A diagnosis of hypoglycaemic coma with a background obstructive uropathy was made.

Result of investigations shows random blood sugar to be 1.9mmol/L, serum electrolyte, urea and creatinine were within normal limits, Haemogram was 16.0g/dl and the PSA was 57.0ng/ml.

He had bolus intravenous administration of 50ml of 50% dextrose, in double dilution, and he regained consciousness; and he was maintained on 10% dextrose in water with 6hourly clinical and blood glucose monitoring. The fasting blood glucose three days later was 4.2mmol/L. He had prostate biopsy twice, on account of elevated PSA, and histology report was consistently benign prostatic hyperplasia. He had retro-pubic prostatectomy and post – operative period has being uneventful so far.

Case 2

A 63-years-old farmer was admitted through the emergency unit of the hospital with a diagnosis of hypoglycaemic coma. He had difficulty with voiding urine and this has being on for about one year period and characterized by worsening LUTS but no urinary retention. He had occasional initial haematuria but there was no passage of clot or necroturia. There was no previous history of trauma, urethra instrumentation or childhood haematuria. He also had recurrent fever with fixed right loin pain. His symptoms worsen in the few months prior to presentation when a friend who had 'similar' symptoms "prescribed" to him some pills that had "worked for him" (Daonil[R] [Glibenclamide]) following which he went unconscious some hours later. He is not a known diabetic, hypertensive and no surgery in the past.

He was admitted with a Glascow Coma Score (GCS) of 10. He was mildly pale, anicteric with good hydration. The chest and cardiovascular findings were essentially normal. The significant finding on abdominal examination was a moderately enlarged, hard and nodular prostate with obliteration of the median sulcus.

A diagnosis of drug-induced hypoglycaemia with suspected carcinoma of prostate was made.

The random blood sugar at admission was 2.0mmol/L, there was azotaemia and the PSA was more than 100ng/ml. The abdomino-pelvic USS shows moderate calyceal fullness of the kidneys, an enlarged prostate with distorted echo texture and thickened bladder wall. He had bolus intravenous administration of 50ml of 50% dextrose, in double dilution and he regained consciousness and was maintained on 10% dextrose in water with 6hourly clinical and blood glucose monitoring. The fasting blood sugar some days later was 5.0mmol/L.

Histology of prostatic needle biopsy confirmed adenocarcinoma of the prostate (Gleason score = 7). He opted for anti-androgen (flutamide) but he was neither regular on the medication nor on his follow-up. His symptoms progresses with severe back pain radiating to the lower limbs, bilateral pedal oedema and bleeding per rectum; at this point he consented for orchidectomy which he subsequently had. He did well immediate post operatively as evident by both biochemical and clinical response; he has been loss to follow up.

Case 3

A 65-year-old farmer was referred from a private hospital after been resuscitated for drug-induced hypoglycaemic coma. He had preceding history of LUTS that became worsened 6-months prior to presentation after failing all traditional medications. A friend who had 'similar' symptoms however introduced him to Diabinese(R) (chlorpropamide) which he took; he went unconscious after some hours following the drug administration. His random blood sugar could not be ascertained from the referring hospital but the FBS few days later was 4.8mmol/L and the glycosylated haemoglobin was 5.4%.

At presentation, he was awake, alert and fully conscious. The chest and cardiovascular systems were essentially normal but had some hearing impairment in the right ear. Prostate is clinically enlarged and benign. Ultrasound confirmed an enlarged prostate with

thickened bladder wall but preserved upper urinary tract. He had open retropubic prostatectomy and he did well at follow up.

Case 4

A 55-year-old policeman was referred from the police clinic on account of suspected hypoglycaemic coma following a fall on his way out of toilet. He was said to have taken glibenclamide which he claimed to have been 'prescribed' to him by his friend. He had history of LUTS which has worsened in the last six months prior to presentation.

He was admitted with Glasgow Coma scale (GCS) of 3, not pale, no facial paresis and no sign of meningeal irritation. The blood pressure at admission was 150/90mmHg. There was suprapubic fullness about 22 week size bladder and failed urethral catheterization to empty the full bladder.

A diagnosis of hypoglycaemic coma with urine retention was made. The blood sugar at admission was 1.1mmol/L, the packed cell volume was 28% and the urea and creatinine were elevated (16.4mmol/L and 256umol/L respectively). The prostate was enlarged with thickened bladder wall and moderate bilateral hydronephrosis on ultrasound.

He had 50ml of 50% dextrose in double dilution, with improvement in the conscious level, and maintained on 10% dextrose in water. The fasting blood sugar was 5.4mmol/L and the glycosylated haemoglobin was 3.8% (normal range of value 6-8%). He also had suprapubic cystostomy to relieve retention following failed urethral catheterization. He later had retrograde urethrogram that confirmed urethral stricture for which he had urethroplasty.

Case 5

A 60-year-old farmer was admitted into the emergency unit of our institution on account of altered state of consciousness following suspected ingestion of oral hypoglycaemic medication. He later volunteered that he had ingested Diabinese[R] which was given to him by his friend after he complained of his abnormal pattern of urination to him. He had being coping with some bothersome LUTS for well over one year prior to this incidence.

At presentation he was not febrile, anicteric well hydrated but was in a state of altered level of consciousness (GCS was 7 at admission). The prostate was enlarged with benign features.

The admitting random blood sugar was 1.4mmol/L, prostate was about 80g by ultrasound and the PSA was 4.5ng/l. in addition, the glycosylated haemoglobin was 4.2%; which was within normal limit.

He had bolus intravenous administration of 50ml of 50% dextrose, in double dilution and he regained consciousness and was maintained on 10%

Table 1: Showing the characteristics of the patients presenting with drug-induced hypoglycaemia

	age (yrs)	sex	occupation	presentation	GCS	drug ingested	source of drug	RBS (mmol/L)	FBS (mmol/L)	glycosylated Hb (%)	treatment
C1	65	male	business	unconscious	8	chlorpropamide	friend	1.9	4.2	NA	50% dextrose
C2	63	male	farmer	altered state of consciousness	10	glibenclamide	friend	2.0	5.0	NA	50% dextrose
C3	65	male	farmer	referred, after been resuscitated	15	chlorpropamide	friend	NA	4.8	5.4	Nil
C4	55	male	police	unconscious	3	glibenclamide	friend	1.1	5.4	3.8	50% dextrose + SPC
C5	60	male	farmer	altered state of consciousness	7	chlorpropamide	friend	1.4	5.0	4.2	50% dextrose

C = case series, GCS = Glascow Coma Score, RBS = Random Blood Sugar, FBS = Fasting Blood Sugar, NA = Not Available , Hb = Haemoglobin, SPC = suprapubic cystostomy.

Table 2: Showing the urological symptoms, diagnosis and treatment offered.

	age (yrs)	sex	occupation	nature of urologic symptoms	duration of symptom	final urologic diagnosis	treatment offered	outcome
C1	65	male	business	LUTS	2 years	BPH	prostatectomy	alive
C2	63	male	farmer	LUTS, haematuria	1 year	CaP	BSCO	alive
C3	65	male	farmer	LUTS	1 year	BPH	prostatectomy	alive
C4	55	male	police	LUTS	6 months	USD	urethroplasty	alive
C5	60	male	farmer	LUTS	1 year	BPH	prostatectomy	alive

LUTS = lower urinary tract symptoms, BPH = benign prostatic hyperplasia, CaP = cancer of prostate, USD = urethral stricture disease, BSCO = bilateral subcapsular orchidectomy.

dextrose in water with 6hourly clinical and blood glucose monitoring. The FBS at a later date was 5.0mmol/L. He was subsequently further evaluated and had open retropubic prostatectomy, he did well post operatively.

Discussion

We described case series of five consecutive patients who presented with drug-induced hypoglycaemia, from misuse of OHA to treat LUTS, seen over a period of eight years. Although, international comparisons of the prevalence of drug misuse are difficult due to differences in data collection and analysis, there is paucity of data from sub-Saharan African until the early 1980s[5]. Oral hypoglycaemic agents were rarely included among the list of commonly abused or misused substances; and of particular interest is their use among patients with LUTS[5,6].

Five tested patients (C1-C5) [Table 1 & 2] had laboratory confirmation of hypoglycaemia that was induced following the misuse of oral hypoglycaemic agent (OHA). All the patients were in 5th and 6th decade of life, they all had LUTS that necessitated the misuse of OHA that was introduced to them by their friends (C1-C5). They all presented in varying state of altered consciousness soon after they had ingested the drug except for one (C3); who was referred to our unit after been resuscitated for suspected hypoglycaemic coma at a peripheral hospital.

Three of them were farmers (C2, C3 & C5) while one each was businessman (C1) and civil servant (C4). Except for the case that was resuscitated at the peripheral hospital, all others presented with Glascow Coma Score that varies from 3-10. The random blood sugar (RBS) obtained from them at the time of presentation ranges between 1.1mmol/L and 2.0mmol/L; four days after treatment and stabilization the fasting blood sugar (FBS) obtained from them ranges from 4.2mmol/L to 5.4mmol/L (confirming non-diabetes state). The corresponding glycosylated haemoglobin (HbA_{1C}) that was estimated in the three later patients (C3, C4 & C5) ranges from3.8% to 5.4%, also within non-diabetes state.

All the patients had 50ml of 50% dextrose in double dilution and later maintained on 10% dextrose infusion with 3 hourly clinical and blood glucose monitoring. In addition, urethral catheter was passed to relieve urinary obstruction and also monitor the patients' urine output; however, C4 had suprapubic cystostomy following failed urethral catheterization to relieve urine retention. They all regained consciousness after glucose infusion treatment.

Three of the patients (C1, C3 & C5) had benign prostatic hyperplasia being responsible for their LUTS while the other two had cancer of the prostate (C2) and urethral stricture (C4) respectively. They had retropubic prostatectomy, orchidectomy and urethroplasty respectively and are doing well at the last follow up.

These patients, as expected, presented to the medical emergency with the classical Whipple's triad of symptoms related to low blood sugar, low blood sugar confirmed at the time of symptom and improvement when blood sugar was restored to normal[1]. The high index of suspicion of drug misuse was made possible because of the classical presentation and the fact that the patients were not known diabetes on drug. They were all given or introduced to the OHA by their friends who are probably being managed for diabetes. The confusion in their symptoms might have arisen due to the confusion of frequency of micturation that is a component of LUTS with the polyuria that comes with diabetes.

All the patients had on-the-spot estimation of RBS which was consistent with hypoglycaemia, the FBS was estimated within three and four days after presentation and when the patients have been stabilized. In addition, three of them also had estimation of glycosylated haemoglobin to establish the blood glucose status in the previous 2-3 months[7]. When compared with the recommended guidelines in evaluation and management of hypoglycaemia in persons without diabetes mellitus our evaluation was limited because, all the patients had never had similar experience in the past and the index hypoglycaemia was drug-induced[1]. During follow up period, none of them had similar incidence before and after treatment for the cause of their LUTS.

All our patients routinely had 50ml of 50% dextrose as bolus double-dilution infusion and maintained on 10% dextrose infusion every 8 hours for the next 24 hours. This was equivalent to 25 grams of carbohydrate at the acute intervention phase to reverse the severe hypoglycaemia and prevent permanent neuronal damage and mortality. The maintenance dextrose infusion is to compensate for the normal daily requirement of 100 grams of glucose and the intracellular deficit. The subsequent measure to search for and treat the underlying cause of hypoglycaemia was not undertaken in our patients since the cause was due to the drug misuse. In addition, they were evaluated for the respective cause of their LUTS and treated according to the pathology found.

Although, no residual morbidity or mortality was documented in these patients, the menace of uncontrolled drug dispensing needs to be checked to preventing morbidity and possible mortality that can be associated with this practice. The healthcare providers need to educate their client properly on the use of their drugs and that the medications are strictly for them and may be dangerous for any other person; even with similar symptomatology.

The onus is on the relevant agencies of the

government to ensure strict compliance with the rules and regulations that governs prescriptions and dispensing of drugs.

Conclusion

The misuse of OHA by patients to treat LUTS with subsequent presentation in hypoglycaemic coma should be of concern to the health care provider especially the urologist. It is of no benefit and constitutes drug abuse that should be discouraged by insisting that the drugs are for individual patient only when drugs are prescribed.

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Anatomical Practices Of Preserving, Handling And Management Of Human Remains: A Proposition For An Act In The Laws Of Nigeria

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Abstract

The anatomical practices of handling, managing, preserving and disposing human remains should be adequately regulated. It is not uncommon to observe poor management of human remains especially with regards to preservation, handling, treatment and transportation. Deaths resulting from disasters as well as some unwholesome practices are major factors that affect the quality of practices. Yet, the management of human remains requires utmost attention for the purpose of health, ethics and environment. Outbreaks of diseases and the spread of such, for instance, the case of Ebola virus, in Liberia and Guinea are obvious pointers to the need to pay adequate attention to the management of human remains. Though this was originally partly addressed by the Anatomy Act of 1933, currently found in the Laws of the Federal Republic of Nigeria; changes and advancements in societal life and circumstances have however limited the practicability of some aspects of the old Anatomy Act, especially as it relates to the management of human remains for non-dissection educational purposes. To this end; a conscientious review of the old act relative to the current scenarios was carried out with the aim of proposing an effective system of taking care of human remains. The result of this review is that an act will be required to address the management of human remains as well the regulation of the anatomical practices of preserving, handling and management of human remains. Human remains as used in this context includes established lifeless or dead whole human body otherwise known as corpse or cadaver, or dead organs and tissues that require disposal.

Key Words: anatomical practices, human remains, mortuary and funeral homes, act

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Introduction

The anatomical practices of preserving, handling and management of human remains requires proper supervision for a number of reasons, especially the following:

1. *Health and Safety*
2. *Professionalism and Quality Assurance*
3. *Ethics and Culture*

Historically, the Anatomy Acts made provisions for handing cadaveric bodies for the purpose of Anatomical examinations and education such as dissection and prosections. The tissue act was developed from such documents in some countries to meet the need of handling of human tissue and remains [1,2]. Some countries have modern laws to specifically address the management of cadaveric human remains meant for disposal or interment [3]. Nigeria, however is yet to have a law to specifically address the establishment of mortuary and funeral homes and regulation of the anatomical practices of preserving, handling and management of human remains. *The closest to the current proposition is section 6-9 of the Anatomy Act of 1933 which however only addressed anatomical examination and disposal of such bodies. Even the original version of the English Law;* The Anatomy Act of 1832, passed by the British government, regulated the supply of cadavers for medical research and anatomy teaching was a model upon which some others, most likely the Nigerian anatomy act were premised. Although, the Anatomy act only addressed the use of cadaveric bodies for anatomical demonstrations and medical education purposes; the more relevant to the current proposition, as a good example, is the Law on the Burial of Human Remains of the Republic of Lithuania.[3]

It is important to emphasize the fact that proper and hygienic anatomical practices are greatly required when managing human remains. The possibility of the spread of infectious diseases, which might have been the cause of death, is a major consideration for any individual saddled with this responsibility. The case of Ebola outbreak in the West African region [4] is a popular instance of the need to properly manage human remains. In addition, the possibility of improper disposal of body fluid and tissue from such human

remains is another major consideration. This might also pose risks not just to human health, but also to the non human inhabitants of the environment[5].

Another important consideration when handling and managing human remains - whether as a whole or parts due to the nature of death - should be professionalism and quality of practices. The level of knowledge and skills of the individual(s) in charge of such responsibilities will nevertheless influence their choice of methods and practices to preserve, handle, store or transport human remains among others. To this end; it will be very important to regulate this practice(s) towards ensuring professionalism and quality assurance.

Methodology

This proposition looked into three vital considerations that are associated with international standard in the management of human remains:

1. Health and Safety: This should consider the health and safety of the personnel who carries out the processing and management of human remains; the health and safety of other humans in the environment and the health and safety of other non-human member of the environment.

2. Professionalism and Quality Assurance: This should consider the deployment of adequate techniques and methods which are possessed by trained and certified individuals saddled with such specific responsibilities.

3. Ethics and Culture: Ethical issues and considerations should be central to the use of techniques, technology and methods; as such, there should be adequate consideration for ethics. Also, the cultural practices should be considered as long as they do not compromise the workability of the first two considerations.

To this end the proposition would employ two key strategies to achieve the above:

1. Establishment of mortuary and funeral homes
2. *Regulation of the Anatomical Practices* of preserving, handling and management of human remains.

The result of the above consideration is the development of the current proposition for the act

The Proposition

(An ACT to provide for the establishment, control and regulation of mortuaries and funeral homes; and to provide for the disposal of corpses and the interment of human remains, in a dignified manner; and to provide for matters connected therewith)

1. Short title

This Act should be cited as MORTUARY AND FUNERAL HOMES ACT, 2016

2. Application of the Act

The Act should apply to any private or public mortuary or funeral undertakers' premises, including those under the control of any Government Department, such as the police services and public hospitals.

3. Implementation of the Act

(1) The Act should be implemented by a regulatory body for the school and practice of anatomy in Nigeria (provision should be made for this by amending the current Anatomy Act to establish the regulatory body, Anatomy Council of Nigeria)

(2) Such Council should make rules and regulations to guide establishment, control and regulation of mortuary and funeral services in Nigeria and should be responsible to the Minister of Health

4. Granting permission to render mortuary and funeral services

(1) Save under and in accordance with a permission granted by a Director of Anatomical Services in a State Ministry of Health, no person should carry on the business of mortuary and funeral services.

(2) Permission by such Director of Anatomical Services in any State Ministry of Health should be granted to a certified anatomist or anatomy technician who must be licensed to practice anatomy in Nigeria

(3) Every such permission should be given to certify the mortuary halls or funeral undertakers' premises in form of a certificate of competence and should be valid for a period of 2 years from the date of issue.

(4) The grant or renewal of any such certificate of competence should be subject to the payment in advance to such Director of Anatomical Services a prescribed fee.

(5) Where such Director is satisfied that any such certificate of competence has been lost or destroyed, he may, upon payment of another prescribed fee, issue a duplicate thereof.

5. Refusals and revocations

Such Director of Anatomical Services may refuse to grant or renew a certificate of competence, or may revoke a certificate of competence granted, to a holder of such premises if he is under the age of 18 years or if, in the opinion of a directorate of anatomical services in the state ministry of health, such refusal or revocation is in the public interest.

6. Requirements as to posting of licensees'

names, etc
Holders of mortuary and funeral homes should cause his name and the words "Mortuary and Funeral Home" to be posted, in English lettering not less than 100 mm in height, in a conspicuous place on the outside wall or door of the premises to which his certificate relates.

7. Issue of a certificate of competence
(a) Being subjected to the provisions of such Act we are proposing, no person should prepare or store any human remains except on approved funeral undertaker's premises or mortuary halls in respect of which a certificate of competence should have been issued by a Director of Anatomical Services in a State Ministry of Health, and is in effect.

(b) A directorate of Anatomical Services in a State Ministry of Health may, if it is satisfied that health nuisance exists on any funeral undertaker's premises or mortuary, situated in its state, issue a written order to the owner or person in charge of the premises in question to stop all activities connected with the handling, preparation and storage of human remains on the premises, until such time that the health nuisance referred to, in the order has been eliminated.

8. Application for the issue of a certificate of competence
(a) Any person wishing to apply for a certificate of competence in respect of new funeral undertaker's premises or mortuary should, not less than 21 days before submitting his application to the state ministry of health, cause a notice to be published in a newspaper, which circulates in the area in which such premises are situated.

(b) Such notice should contain information to the effect that an application for the issue of a certificate of competence in terms of these regulations is to be submitted to a directorate of anatomical services in a state ministry of health, and that any person who would be affected by the use of such funeral undertaker's premises or mortuary and wishes to object to such use should lodge his objection, together with substantiated representations, with such directorate of anatomical services in a state ministry of health in writing within 21 days of the date of publication of such notice.

(c) An application for the issue of a certificate of competence should be made in writing by the applicant or his authorized representative to such directorate of anatomical services in a state ministry of health in whose area of jurisdiction the funeral undertaker's premises or mortuary is located, on such form as prescribed directorate

(d) An application for the issue of a certificate of competence should be accompanied by
(i) a description of the premises and the location thereof;
(ii) a complete ground plan of the proposed construction or of existing buildings on a scale of 1:100;
(iii) a block plan of the premises on which true north is shown indicating which adjacent premises are already occupied by the applicant or other persons and for what purpose such premises are being utilized or are to be utilized; and
(iv) particulars of any person other than the holder or any of his employees who prepares or will prepare human remains on the premises.

(e) A directorate of anatomical services in a state ministry of health, when considering issuing a certificate of competence, may request from the applicant or any other person any such further information as it may deem necessary to enable it to properly consider the application concerned.

(f) No directorate of anatomical services in any state ministry of health should consider any application for the issue of a certificate of competence unless a complete inspection of the premises concerned has been carried out by an Environmental Health Practitioner, and his/her report and recommendations on such inspection are available to such directorate of anatomical services.

9. Issue of certificate of competence
Where a directorate of anatomical services, after consideration of an application for the issue of a certificate of competence, the report concerned by a Environmental Health Practitioner including his/her recommendation, and any objections to the use of funeral undertaker's premises or mortuary, is satisfied that the premises or mortuary concerned (a) comply with all requirements laid down in this Act; (b) are in all respect suitable for the preparation of human remains; and (c) will not be offensive to any occupant of premises in the immediate vicinity of such premises, the Director of Anatomical Services in such state ministry should issue a certificate of competence in the name of the holder in such form as it may be determined.

10. Issue of provisional certificate of competence
(1) Notwithstanding the fact that a directorate of anatomical services is not satisfied as contemplated in section 7, with regard to funeral undertaker's premises or mortuary in respect of which a certificate of competence might have been applied for, such directorate
(a) should in the case of existing funeral undertaker's premises or mortuary; and

(b) may, in all other cases, subject to such conditions as such directorate of anatomical services may determine, in general or in each specific case, issue a provisional certificate of competence in respect of such premises for a maximum period of only 6 months to enable the applicant to render the premises in such a manner as to comply with the provisions of this Act, Provided that the directorate shall satisfy itself that the use of such funeral undertaker's premises or mortuary does not and will not create a health nuisance or endanger human health.

(2) A provisional certificate may not be extended unless the directorate of anatomical services concerned is satisfied that the owner or representative thereof is in the process of making the necessary changes as prescribed in sub-section (1) above.
(3) Any such extension in sub-section 2 above will be granted for a period of not more than 12 months.

11. Duties of holder
(1) The certificate holder shall immediately inform the issuing government in writing, if there are any changes in the particulars supplied to the issuing government in the application for the certificate of competence concerned.

(2) Failure by the holder or a person in charge/authorized person to comply with this regulation shall constitute an offence.

12. Corpses to be prepared only at funeral undertaker's premises in respect of which a certificate of competence has been issued
(1) Unless otherwise provided for in this Act, no person shall prepare and/or store any corpse except on a funeral undertaker's premises or mortuary hall in respect of which a certificate of competence has been issued and is in effect.

(2) No dead body shall be embalmed or prepared for burial or encoffining in any mortuary and funeral home otherwise than in a room used exclusively for such purpose.

(3) At all times during which any dead body is retained in any such room, the windows thereof shall be kept fully opened and, if the room is ventilated wholly or partly by mechanical means, the ventilating system shall be kept in operation, but where an air-conditioning system is installed which services such a room, the windows thereof, shall be kept closed and the air-conditioning system shall be kept in operation.

13. Reception and retention of human remains
(1) No dead body in an advanced state of decomposition shall be received into any mortuary and funeral home unless encoffined in a hermetically sealed coffin.

(2) Where it appears to the owner of any mortuary and funeral home that the body of any person who has died while suffering from any quarantinable disease has been received into such mortuary and funeral home, he shall forthwith notify the directorate of anatomical sciences which shall make a case of such to the appropriate directorate of the state ministry of health.

14. Hygiene requirements for funeral undertaker's and mortuary premises
(1) All solid waste on the premises of a funeral undertaker or mortuary shall be kept in corrosion-resistant and rodent proof containers with tight-fitting lids and shall be dealt with in accordance with the solid waste management requirements of the Federation.

(2) Every holder of a certificate of competence or provisional certificate of competence for funeral undertaker's premises or mortuary shall ensure that
(a) employees and all other persons involved in handling of human remains are provided with clean and appropriate protective clothing consisting of surgical gloves, gumboots, plastic aprons so designed that the front hangs over the top of the gumboots, face masks and linen overcoats, and each such employee or other person shall, at all times when so involved, wear such clothing;

(b) premises are kept free of insects, offensive odours, gases and fumes;

(c) all working areas or surfaces at such premises where human remains are prepared are cleaned and disinfected immediately after the preparation of any human remains;

(d) cause all equipment used for the preparation of human remains to be washed and disinfected immediately after use;

(e) cause all used protective clothing to be washed, cleansed and disinfected daily on the premises;
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(f) if a human remain has been transported without a moisture-proof covering, cause the loading space of the vehicle concerned to be washed and disinfected after such human remains has been removed; and

(g) the number of human remains (corpses) kept within the premises shall not exceed the number of removable trays available to accommodate such human remains (corpses) in the refrigerators or cold chambers.
(3) All waste generated in the preparation room shall be

deemed to be health risk waste and the collection, storage, handling and disposal of as such waste shall be done in accordance to relevant health care risk waste standards.

15. Unclaimed bodies or unidentified human remains
Any unclaimed bodies or unidentified human remains must be dealt with in accordance with the provisions of the Regulations Regarding the Rendering of Forensic Pathology Service.

16. Appeals
(1) A person affected by a decision taken in terms of this Act who wishes to appeal against the decision, must lodge an appeal with the Anatomical Council of Nigeria through the directorate of anatomical services in his/her state ministry of health, as the case may be, within 30 days after that person has been notified of the decision.

(2) The Registrar of the Council shall after considering all relevant information make a decision and inform the appellant of such decision.

(3) Reasons for the decision must be provided to the appellant in writing.

17. Offences and penalties
(1) Any person who
(a) contravenes or fails to comply with any provisions of this Act;

(b) contravenes or fails to comply with any notice given or condition imposed in terms of this Act

(c) for the purpose of this Act, makes a false statement knowing it to be false or deliberately furnishes false or misleading information to an authorized official or officer;

(d) threatens, resists, interferes with or obstructs an authorized officer or employee for the Council in the performance of his powers, duties or functions under this Act, shall be guilty of an offence and upon conviction

(2) Any person who is guilty of an offence and is convicted shall be liable to a fine or to imprisonment for a period of not less five years or to both a fine and such imprisonment.

18. Registers
(1) The owner of any mortuary and funeral home shall keep a register in which he shall correctly record the following particulars

(a) the name, age, sex and address of the deceased;
(b) the date, place and cause of the death of the deceased;

(c) the date on, and the time at, which the remains of the deceased were received into the mortuary and funeral home;

(d) the date on, and the time at, which such remains were removed from the mortuary and funeral home;

(e) the date and number of the death certificate and of any permit for the burial, importation into or removal from Nigeria, as the case may be, of such remains;

(f) the name and address of the medical practitioner (if any) who certified as to the death of the deceased, and of the person signing any permit for burial, importation into or removal from Nigeria, as the case may be, of such remains;

(g) the name and address of the person (if any) who has undertaken to pay the charges (if any) of the mortuary and funeral home;

(h) where the deceased died from any quarantinable disease, whether or not those parts of the mortuary and funeral home in which such remains were kept were disinfected after such remains were removed therefrom.

(2) The owner of mortuary and funeral home shall cause the register kept by him under subsection (1) to be open for inspection at all reasonable hours by any public officer authorized in writing by the Director of Anatomical Services from the State Ministry of Health, for the purposes of this section, or any health officer, health inspector or police officer.

19. Conveyance of human remains
(1) The human remains of a person who, at the time of his or her death suffered from a disease or condition which is capable of transmitting an illness even after death and in the opinion of the health authority concerned, may pose a health hazard or endanger public health in one way or another, may not be conveyed in public in any way unless

(a) such human remains are placed in a polythene bag, sealed in an airtight container, placed in a sturdy non-transparent sealed coffin, embalmed and/or the total surface of the body covered with a 5 cm layer of wood sawdust or other absorbent material which is treated with a disinfectant;

(b) a medical practitioner declares in writing that in his or her opinion the conveyance of such human remains will not constitute a health hazard;
(c) such declaration must accompany the human

remains at all times during the conveyance and up to the burial; and

(d) for human remains of a person whose cause of death was a communicable disease, the body shall not be embalmed, but strict guidelines on management of communicable diseases as may be published by the Federal Ministry of Health shall be followed.

(2) The declaration referred to in sub-section 1 shall be shown to an officer on demand by the person responsible for the conveyance of the human remains.

(3) No person shall damage a container referred to in sub-section (1) (a), or open such container or remove the human remains from the container or come into direct contact with the human remains after it has been sealed without prior approval from an officer referred to in sub- section (1)(b).

20. Requirements for the transportation of human remains inland and across the borders of the Federal Republic of Nigeria
(1) No person shall convey any human remains in any manner that may endanger public health.

(2) Any person transporting any human remains from the country across an inland border to an area outside the country or to the country across an inland border from an area outside the country shall ensure

(a) in the case of public transportation, that the human remains are sealed in an airtight container and placed in a non-transparent, sturdy, sealed coffin; or

(b) in any other way, that the human remains have been placed in an approved coffin or container.

(3) Any human remains unloaded or received from a point of dispatch or loading point outside the country shall be transported in a manner consistent with sub-section (2)

(4) No coffin or container in which the human remains have been placed may be conveyed using public transportation unless
(a) the outer surface of such coffin or container is free from any leakages or any other secretion matter emanating from such human remains; and

(b) any offensive odors are eliminated.

(5) The person responsible for the conveyance of a human remains shall, at all cost ensure that should any leakages, secretions or odors emanating from the container of the human remain conveyed, such coffin or container is taken forthwith to the nearest mortuary or undertaker's premises, where the necessary measures shall be taken to eliminate such conditions.

(6) The provisions of sub-section (1) shall not apply to the human remains of a person
(a) who died in the country and whose human remains are intended for burial in an area outside the country in cases where an authority in that area, who has direct jurisdiction over the application of health measures in that area, authorizes, in writing, the bringing of such body into that area on conditions other than those prescribed by sub-section 1; or

(b) who died outside the country and whose human remains are intended for burial in the country in cases where a medical officer of health or other medical practitioner in the employ of the state is of the opinion that the bringing in of such human remains will not constitute a danger to health in the country or part thereof; and provides written authorization for such body to be brought into the country.

21. Authorization to import and export human remains
(1) Subject to the provisions of regulations 13 above, no person may import or export human remains unless he or she is issued with an import or export permit by the Director of Anatomical Services of the State, such import or export permit shall be valid for a period of 30 days after the date of issue.

(2) Should any human remains be imported into the country in which an import permit as set out in sub-section (1) has not been issued, the Director of Anatomical Services of the State may order that such human remains be kept in a mortuary or at an undertaker's premises at the expense of the importer until such time that the required permit has been issued, provided that if the prescribed permit is not issued within 30 days after the date of the order, the Director of Anatomical Services of the State may order that such human remains be buried or dealt with in accordance with the burial prescripts in the country and such burial shall be at the expense of the importer.

(3) Any person requiring an import permit referred to in sub-regulation (1) shall apply to the Director of Anatomical Services of the State in writing and such application shall be accompanied by the following:

(a) a death certificate, indicating the deceased's name, address, the date and place of death and the cause of death.

(b) the name and export permit of the country from which the human remains are to be imported;

(c) the name of the first point of entry where the human

remains is to be imported, the type of transport to be used to import the human remains and convey it to the place of burial;

(d) the name of the place in the country where burial of the human remains is to take place or if the human remain will not be buried the reason why the human remains is being brought in; and

(e) an embalming certificate, except for deaths as provided for in sub-section 3 and in specific cases where embalming is prohibited for religious reasons.

(4) The provisions of sub-section (3) (a) should *mutatis mutandis* apply to the human remains of a person that has died:
(a) in transit on a boat or aircraft the moment that the human remains is being brought into the country, irrespective of whether or not such human remains is to be buried in the country; or

(b) in the country and has to be exported out of the country for compliance with the requirements of the International Health Regulations to contain events at their sources.

(4) The person responsible for the conveyance or burial of human remains should have in his possession the import permit referred to in sub- section (1), such permit should be produced on request by a Port Health Officer at the port of entry in terms of the International Health Regulations.

Conclusion And Recommendation

Generally, human life deserves utmost respect; also, human remains should be treated with regard for ethics and good cultural practices. The consideration of cultural practices could however be controversial as some of these might not encourage safe and hygienic practices in term of health and environmental safety [6]. Again, this was found to be a major problem while tackling the spread of the Ebola virus in the West African countries. To this end, a formal line has to be drawn between ethics, culture and standard practices. Hence, the standard practices, having considered ethics and quality cultural practices would make provisions for best practices and methods of managing, handling, preserving, keeping, storage, transportation or disposal of human remains.

It is therefore important to note that these practices are currently provided by the operator of mortuary and funeral home operators- private and public. As such, what is required is regulation and enforcement of standard practices under the statutes of the law. This is the primary aim of this proposition. It is in line with global best practice and treads in modern societal settings. In line with global trends, vital considerations with respect to the regulation of embalming include: Persons Performing Embalming; Licensing of Embalming; Use of Human Remains for Educational Purposes; Procedure for Embalming of Human Remains; Refusal to Perform Embalming. Also, the regulation of funeral services will require he consideration of the following: Requirements for the Provision of Funeral Services; Rights and Duties of the Person in Charge of Funeral Arrangements; Transportation of Human Remains [3].

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Recurrent Herpes Simplex Virus Keratitis In A Young Nigerian Male Adult: A Case Report.

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Abstract

Herpes Simplex Virus Keratitis is the infection of the cornea by double stranded DNA Viruses.This condition has a tendency to reoccur after a certain period of time. A 25 year old male presented to the clinic with reduced visual acuity, itching, redness, pain and photophobia in the left eye. Onset of symptoms was 10 days prior to presentation. The patient's oculovisual history revealed a previous infection that resulted in scar formation on the cornea of the left eye. A comprehensive case history and slit lamp examination revealed the presence of dendritic ulcer in the left eye of the patient. The patient was diagnosed with recurrent herpes simplex virus keratitis. An aggressive multi-treatment plan involving the use of antiviral, antibiotics, and anti inflammatory drugs was administered to the patient. This was done in order to minimize corneal scarring in the left eye. A significant improvement in the vision of the left eye of the patient was observed after five times of follow-up visitation to the clinic.

Keywords: *Herpes Simplex Virus, Keratitis, Dentritic ulcer, Young adult male*

Introduction

Herpes simplex virus keratitis is a viral infection of the corneal epithelium and stroma. It is caused by herpes simplex viruses (HSV), which are double stranded DNA viruses that affects humans.There are two major types of herpes simplex virus, they are herpes simplex virus type 1 (HSV1) and herpes simplex virus type 2 (HSV2). HSV 1 and HSV 2 belong to the family of herpes viridae, including varicella zoster and human herpes virus types 6-8[1,2] . HSV-1 commonly affects the mouth and eyes while HSV-2 affects the genitals[3]. Majority of HSV keratitis occur as a result of secondary infections that are present after the virus from a primary oral labial infection becomes reactivated in the trigeminal ganglion and extend to the eye through the ophthalmic branch of the fifth cranial nerve.

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Risk factors for reactivation are thought to include fever, immunosuppression , nutrtional deficiences, toxins, medications, stress, illness, menses, sun exposure and trauma[4].Signs and symptoms of HSV keratitis include pain, excess tearing, foreign body sensation, blurred vision, red eyes, eye lid swelling, corneal edema and corneal scarring. HSV keratitis can reoccur and ultimately lead to corneal surface disease, corneal scarring, neurotrophic keratopathy, corneal perforation and blindness in severe cases[1,2]. Chances of reoccurence is greater if the first two episodes are closer together[5]. Also if the first episode of HSV keratitis was severe there is a greater chance that the reoccurring episodes would be severe[5]. The major factors which dictate the severity of recurrent herpes are the viral strain, immune response of the host and treatment.[6] Subsequent reoccurrences can present with larger dendritic ulceration and lesions, chronic dry eyes and conjunctivitis. Patients with a history of HSV keratitis should be counselled about the risk of recurrence.

Case Report

Mr T.K, a 25 year old male student presented to the Optometry Clinic, University of Benin with complaints of itching, redness, pain and photophobia resulting in the left eye. Onset of symptoms was 10 days earlier. The patients' oculo-visual history revealed that he had an infection on the cornea in the left eye about 5 years ago and it resulted in a scar and that he has been wearing glasses for cosmetics, but stopped about 3 months earlier. The patient had used drugs bought from a private pharmacy in an attempt to treat the present condition. The drugs were Gentimicin eye drop, Visine eye drop, Dexamethasone tablets and Ampicillin capsule.The medical history revealed that the patient has just treated malaria with Fansidar.

Clinical Data

Patient's entry visual acuity (VA) for distance was 6/6-3 for OD., near N_5 and 6/ 24 -1 for OS., near N_{18} .Pinhole test revealed visual acuity of 6/12 for OS.
External examination of the both eyes using Shin-Nippon slit lamp revealed no abnormalities in the right eye but detected eyelid oedema, conjunctival injection, characteristic dendritic ulcer staining (evident on fluorescein staining) on the cornea in the 5 and 9 o clock position and flares in the anterior chamber of the left eye.

Internal examination of both eyes with Neitz monocular direct ophthalmoscope revealed no abnormalities for the right eye (cup to disc ratio was 0.3) but could not visualize the interior parts of the left eye.

The patient was diagnosed with recurrent Herpes Simplex Keratitis based on clinical findings, and was placed on various eye drops and tablets. They include

Gutt: Moxifloxacin i hrly LE
Gutt: Homatropine tds LE
Oc: Chloramphenicol nocte LE
Oc: Zovirax 5× dly
Tab: Ciprotab (500mg)1 bd x 10/7
Tab: Vitamin C 1000mg 1 dly x 30/7

The left eye was padded The patient was counselled about the condition and told to strictly adhere to the drug prescriptions. He was also advised to discontinue the use of the medications he got from the pharmacy and to come for daily dressing. He was asked to return to clinic the next day.

1st Follow Up: Entry VA for the left eye was 6/18-2., N_8 The conjunctival was still injected, there was significant fluorescein staining in the 5 and 9 'o clock position on the cornea of the left eye. Examination of the interior part of the left eye was deferred. Other medications were added. They include tab Acyclovir 800mg 5× daily for 10 days, tab Cataflam 50 mg bd for 5 days (immediately after eating). The left eye was not padded due to the presence of discharge but was told to come for padding when the discharge subsides. The patient came two days later for dressing in the left eye.

2nd Follow Up: The patient said his left eye feels better, no pain was present. Entry VA for the left eye was 6/9-1. N_6 There was mild injection on the conjunctival, there was still fluorescein staining in the 5 and 9 'o clock position on the cornea of the left eye. No abnormality was detected during fundus examination. Cup to disc ratio was 0.3. Ciprotab, Cataflam and Moxifloxacin eye drop was exhausted .The patient was told to buy another Moxiofloxacin eye drop (dosage was reduced to six times daily) and to continue with his previous medications and to come for daily dressing.

3rd Follow Up: No new compliant. Entry V A was 6/9-1.There was mild injection on the conjunctiva of the left eye. There was reduced fluorescein staining on the cornea. The patient was advised to continue with his medications and the eye was padded.

4th Follow Up: No new complaints .Entry VA for the left eye was 6/9+1 N_5, there was still mild injection on the conjunctival. There was no fluorescein staining on the cornea but there was faint scar formation on the 5 and 9' o clock position of the left eye. The pad was removed from the left eye. The patient was told to discontinue Homatropine and complete the other medications that were given to him which included OC Zovirax, OC Chloramphenicol, Tab, Acyclovir 500mg.

5th Follow Up: The patient complained about a white spot that was present on the left eye, feels that his vision has improved. Entry VA for the left eye was 6/6-2. Near VA was N_5. There was no injection on the conjunctiva. He was told to come back for refraction.

Discussion

Herpes simplex virus (HSV) is a common cause of corneal disease and is the leading infectious cause of corneal blindness in developed nations[7]. The global incidence of HSV keratitis is roughly 1.5 million, including 40,000 new cases of severe monocular visual impairment or blindness each year[8]. A retrospective analysis conducted on the records of children examined in a teaching hospital in Nigeria revealed 45% of these children had presumed Herpes Simplex keratitis[9]. Mode of transmission of HSV 1 is primarily through direct contact with infected secretions or lesions. Once the virus is present in the tissues, it spreads from the site of initial infection to the neuronal cell bodies, where it can lie dormant for years until reactivation occurs.[10]

HSV Keratitis can be divided into four major categories[2]. The first category is the infectious epithelial keratitis which is made up of corneal vessicles, dendritic ulcers, geographical ulcers and marginal ulcer. The second category is neurotrophic keratopathy and includes punctate epithelial erosions and neurotrophic ulcers. The third category is stromal keratitis which is divided into immune stromal keratitis (due to an immune reaction) and necrotizing stromal keratitis (due to replicating viruses)[2, 11]. A total of 20-60% of patients with recurrent HSV keratitis have stromal keratitis[12-14].The fourth category is endothelitis (due to an active infection) is primarily an inflammatory response to the endothelium.

A comprehensive case history can serve as a guide in diagnosis of HSV keratitis especially if there was a prevous episode of herpes infection that led to corneal scarring. Also a detailed slit lamp examination (with flourescein stain) will reveal a branching pattern of dendritic ulcer which is a common presentation of HSV keratitis. However, it should not be confused with other lesions such as Acanthameoba keratitis, Recurrent corneal erosion, Pseudodendritis and Thygeson superficial punctate keratopathy which have similar presentations .True dendritic lesion will present with arborization and terminal end bulbs. Laboratory tests are not an absolute requisite for diagnosis of

herpes simplex virus infections as clinical features are highly characteristic. However whenever possible, culture should be undertaken to establish a firm diagnosis.[6] Various laboratory tests include Giemsa stain for multinucleated giant cells, immune fluorescence assay for HSV-1 antigen and polymerase chain reaction(PCR) for HSV -1 DNA.[15]

With the recent advancement in topical and systemic antiviral drugs, treatment of HSV keratitis infections has improved in the last two decades. Treatment of patients with herpes simplex keratitis depends on the clinical manifestations, the affected layer and the severity of the disease[16]. The aim of treatment is to minimize damage to the stromal layer and scar formation thereby preventing permanent blindness.

As seen in this case, topical acyclovir(zovirax) is the first line antiviral treatment for HSV keratitis. Acyclovir has poor aqueous solubility and is thus formulated as an ointment. Acyclovir is converted to its active form by viral thymidine kinase and only becomes active as binder of DNA polymerase in infected cells.[17] Application of 3% day can lead to a rapid loss of typical morphological features relatable to HSV infection. Although acyclovir is highly effective against HSV keratitis, it is highly hindered due to blurring of vision after application of the ointment .Ganciclovir 0.15% ophthalmic gel has been proven to be an effective, safe and well tolerated treatment for HSV Keratitis. Local tolerance is better than acyclovir hence increasing patient compliance. It also had a prolonged contact time with the cornea and causes less blurring and stinging.[16]. The ingestion of antiviral drugs such as acyclovir tablets does not really have a large effect on the cornea epithelium but can at least reduce the viral load in the ciliary ganglion and associated nerves Prophylactic antibiotics are administered for HSV keratitis in order to prevent super infections.

Topical steroids should be avoided as much as possible during treatment of herpes simplex keratitis. This was also avoided in this case. The use of steroids interferes with the body's defense mechanism prolonging the course and severity of the disease.[18] However cautious administration of corticosteroids is warranted only for severe cases with prolonged keratitis, relatively dense infiltration, early vascularization and intense iridocyclitis.[19]. Nutritional supplements such as vitamin C, Zinc and Lysine should be obtained from the diet.

Referral to an ophthalmologist is advised if HSV Keratitis infection does not subside within a few weeks of treatment.

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Experience In The Management Of Dandy-Walker Syndrome In An Adult Patient

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Abstract

Dandy-Walker Syndrome (DWS) is a congenital brain malformation involving the cerebellum and the fluid spaces around it. There is atresia of foramenofMagendie and Lushka resulting in a complete absence of the part of the brain located between the two cerebellar hemispheres (cerebellarvermis) and cystic dilatation of the fourth ventricle. It may be associated with other anomalies in the brain and other parts of the body. Symptoms such as delayed developmental milestone, bulging of skull may present early in life or may present later in life. We present a case of DWS diagnosed in adulthood with features of raised intracranial pressure (ICP) (headache, vomiting, neck stiffness, and convulsion) and poor academic performance. Difficult airway was anticipated due to the presence of micrognathia and reduced neck mobility.The patient was managed successfully with cysto-periotoneal shunt insertion under general anaesthesiawithout postoperative ventilation.

Keywords: Dandy-Walker Syndrome, Hydrocephalus, VP-Shunt, Airway, Adulthood.

Introduction

Dandy-Walker syndrome is a rare sporadic genetic disorder characterized by a neuropathological triad comprising of hypoplasia of the cerebellarvermis, cystic dilatation of the fourth ventricle and hydrocephalus as a result of atresia of the exit foraminal of Magendie and Luschka. The syndrome was first described by Dandy and Blackfan in 1916. The syndrome has a slight female predominance and an incidence of around 1 in 25,000-30,000 life births.[1,2]The clinical manifestation usually appear in the first year of life, but can also occur in the neonatal period.[3]

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The symptoms that usually become apparent in early infancy include slow motor development, bulging of the anterior fontanel and progressive enlargement of the skull due to the obstructive hydrocephalus. Associated congenital anomalies may include craniofacial anomalies such as cleft palate, micrognathia, hypertelorism, cardiac, renal and skeletal malformations. Cerebral anomalies include agenesis of corpus callosum with poor intelligence and interference with medullarycontrol of respiration which can lead to respiratory failure. We report our experience in the management of a twenty one year old adult diagnosed with Dandy- Walker Syndrome who had cyto-peritoneal shunt on account of raised intracranial pressure.

Case Report

A twenty-one year old boy weighing 55 kgspresented with history of persistent headache and progressive loss of vision of 2 weeks duration. There was associated neck pain, photophobia and vomiting. He had an episode of tonic clonic convulsion which lasted for about 3 minutesduring which he hit his head on the floor. The seizure aborted spontaneously prior to presentation to the hospital. There was past history of convulsionat3 years of age and was been managed for seizure disorder with phenobarbital. There was no history suggestive of cardiac or renal anomalies. No history of delayed developmental milestone. He was withdrawn from secondary school due to poor academic performance.

Pre-anaesthetic examination, revealed a conscious boy with micrognathia, ptosisof left eye (figure 1) with dilated unreactive pupil and todd's palsy affecting the right upper and lower limb. There was neck stiffness with positive Kernig's sign. Mouth opening was adequate(Figure 2) and has Mallampati II. Haemogram and electrolyte result were within normal range. Brain CT-scan revealed cystic dilatation of 4th ventricle with mild to moderate dilation of other ventricles and agenesis of cerebellarvermis. (Figure3) An assessment of Dandy-Walker syndrome was made with secondary hydrocephalus. Consent was obtained for anaesthesia and surgery and fasting guideline was observed.

Intraoperatively, multi-parameter monitor was attached, peripheral saturation, pulse rate, blood pressure, was monitored. An intravenous access was secured on the left hand using size 18 G cannula. He was pre oxygenated with 100% oxygen for 5min with a

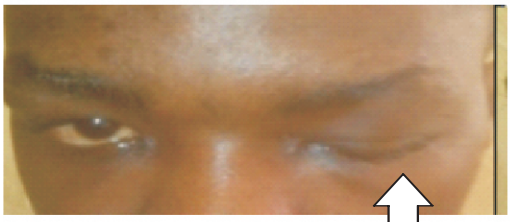

Figure 1

Left eye ptosis

Figure 2

Mouth opening

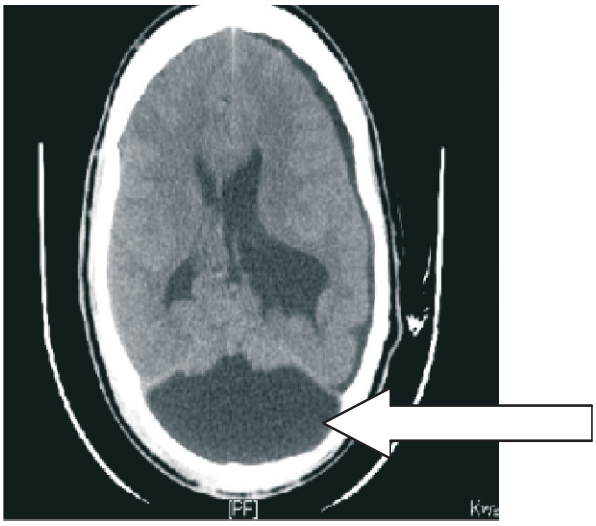

Figure 3

Cystic dilatation of 4th ventricle

tight fitting face mask. Anaesthesia was co-induced with I.V fentanyl 2 µg/kg and propofol 2 mg/kg. Laryngoscopy and tracheal intubation was facilitated with I.V suxamethonium 1 mg/kg.Tracheal was intubated with size 6.5 mm cuffed endotrachealtube and correct tube placement was confirmed with equal air entry on auscultation.

Anaesthesia was maintained with propofol using TIVA, intermittent pancuronium for muscle paralysis, paracetamol and intermittent fentanyl for analgesia. During the course of the surgery, he was haemodynamically stable with SpO2-100%, end tidal CO_2 was maintained between 30 and 35mmHg pulse rate maintained between 70-90/min, and blood pressure between 100-130/60-80 mmHg. Patient had cysto-peritoneal shunt insertion. The procedure lasted for 2 hours 30 min and went uneventful.

At the end of the surgery, patient regained spontaneous respiratory effort. Residual neuromuscular paralysis was reversed with neostigmine 0.05 mg/kg and atropine 0.02 mg/kg. He was extubated after he regained full consciousness and SpO2 of 96-98% in room air. Patient was transferred to recovery room for further monitoring and was maintained on I.V phenytoin sodium postoperatively to prevent seizure.

Patient was discharged home on 4th day postoperatively.

Discussion

The most common clinical presentation of Dandy-Walker syndrome are enlargement of the occiput , macrocrania, mental retardation, cerebellar ataxia and raised intracranial pressure.[4] In older children with Daddy-Walker syndrome, symptoms of increased ICP, including irritability and vomiting, signs of cerebeller dysfunction, including gait disturbance and lack of muscle coordination, may occur.[5]This patienthad been managed as a case of seizure disorder since the age of 3 years with no suspicion of intracranial lesion. The patient presented to our facility with features of raised ICPand worsening neurological status. No radiological evaluation was done probably because of lack of image facility, lack of awareness or ignorance of the managing team since the onset of the symptoms.Accidentally, the diagnosis of Dandy-Walker syndrome was madewith cranial CT scanwhen he was being evaluated for traumatic brain injury after a seizure episode at age 21.

In the present case, the patient had raised ICP and micrognathia. Presence of micrognathiacan make airway management challenging as reported by Ewart and Oh.[6]This patient had mallampati II on airway assessment and he is not at risk of difficult intubation.The important anaesthetic concern in our patient was the management of the ICP and the airway. When difficulties in airway management are anticipated, awake intubation is required despite the increased ICP. In our case, intubation was not difficultdespite the presence of micrognathia, although, difficult airway tray was made available. This finding is similar to that reported by Ji Su Jang et al [5]and Shweta M[7]respectively. Important management strategies should include prevention of further increase in ICP; hence,induction and maintenance of anaesthesia were attained by continuous infusion of propofol which reduced the ICP. End tidal CO_2 pressure was also maintained between 30 and 35mmHg to control the ICP. Intermittent boluses of fentanyl and IV paracetamol were used for analgesia and BP control.In this patient, normocapnia,normotension was also employed to reduce ICP and this is similar to Ramesh et al[8]study.Anaesthesia was maintained with controlled

ventilation, analgesia and muscle relaxants and these has been reported by Kim et al .[9]Endotracheal intubation can be difficult due to the large size head in hydrocephalus and micrognathia. Intubation was made smooth by placement of shoulder roll. Emergence from anaesthesiawas smooth and the patient regained spontaneous respiration soon after surgery and did not need ventilatory support as respiratory pattern was good. Postoperatively convulsion was prevented by maintaining patient on intravenous phenytoin which was converted to oral on the second day postoperatively. He was discharged to out-patient clinic for follow-up on oral phenytoin.

Conclusion

Dandy-Walker syndrome may present outside infant age with increase ICP, attention to anaesthesia details is necessary for successfulmanagement.

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