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ORIGINAL ARTICLE

Implanon sub-dermal implant: an emerging method of contraception in Ilorin, Nigeria

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Implanon, a single rod sub-dermal implant is a relatively new contraceptive which offers long term reversible contraception for women. This study seeks to determine the safety, efficacy and acceptor characteristics of Implanon at the family planning clinic of University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria. This study involves a retrospective review of 88 clients who used Implanon from January 2007 to December 2011 at the family planning clinic of the UITH, Ilorin. Of the 2,456 clients who had contraception during the period, 88 had Implanon giving a 3.6% uptake. The mean age of Implanon users in the study was 33.4 years, no teenager used the method and 72 (81.8%) knew about the method from clinic staff. Women with two living children constituted 29 (33%) of the total users, 78(88.6%) users had at least secondary education, all except one client were married and religion did not influence its use. Twenty two (25%) users had side effects, the commonest being menstrual irregularity in 13(59%) of the participants. Discontinuation rate was 26.1% and the commonest reason for discontinuation was the desire to get pregnant 8(35%). The Pearl Index for Implanon in the study was 0. Implanon is an effective long term hormonal contraceptive appropriate in a wide range of women with tolerable side effect profile but is currently underutilised. Wider publicity, education and access are needed to improve client uptake..
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Keywords: Implanon, implant, sub-dermal, contraceptive, Ilorin, Nigeria

INTRODUCTION

Contraception has been used in one form or another for thousands of years but the 20th century ushered in the era of modern family planning services (Okpere 2007). There are a wide range of contraceptives; Implanon was introduced as a user independent, long term hormonal contraceptive with minimal side effects. Marketing of Implanon was started in 1998 (Association of reproductive health professionals, 2008); it is a single 4 cm long 2 mm wide (Okpere, 2007; Burkman, 2007) rod with an ethylene vinyl acetate (EVA) copolymer core containing 68 mg etonogestrel (Okpere, 2007). The rate of release in the early weeks of insertion is 60-70 μg per day (Affandi *et al.,* 1999), it decreases to about 25-30 μg per day by the end of third year (Organon, 2006) but 25-30 μg per day of etonogestrel is needed to suppress ovulation (Funk *et al.,* 2005). The onset of contraception is within 24 hrs of insertion, the cumulative failure rate is low (Ladipo *et al.,* 2005) and the Pearl index is 0 (Affandi *et al*., 1999, Funk *et al*., 2005).

The mechanism of action involves thickening of cervical mucus, ovulation suppression and suppression of estradiol – induced cyclic maturation of the endometrium (Okpere, 2007, Ladipo *et al.,* 2005). The rod is usually inserted in the non-dominant upper arm using the trocar; its insertion and removal are quicker compared to Norplant and its mean time for insertion is 1.1 minutes and 2.6 minutes for the removal (Mascarenhas, 1998).

Implants can be a good choice for adolescent, women with medical disorders like hypertension or diabetes, anemia, endometriosis or those breastfeeding because progestogen only contraceptive

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like implanon does not increase the cardiovascular risks in healthy young women (MerkiFed, 2008). Its side effects include menstrual abnormalities, emotional lability, weight increase, depression and acne but with a rapid return of fertility when it is removed (Funk *et al.,* 2005, Sergent *et al.,* 2004). It is relatively new compared to other earlier methods in this centre and thus, it is important to evaluate its performance profile. This study therefore seeks to determine the safety, efficacy, acceptor characteristics of Implanon at the family planning clinic of University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria

MATERIALS AND METHODS

Setting

The study was conducted at the Family Planning Clinic of University of Ilorin Teaching Hospital, located in Ilorin, Kwara State of Nigeria. It is a tertiary centre that receives clients from Kwara State and the neighboring states of Kogi, Oyo, Osun and Niger states. The family planning clinic is responsible for providing contraceptive services to clients who present on their own or are referred for such services. Implanon was introduced into the range of services at this centre in 2007. The procedure for Implanon insertion involves extensive pre- and post-insertion counseling as well as further counseling during subsequent clinic visits.

Study design and population

This descriptive retrospective study consisted of all the 88 clients who received Implanon subdermal implant as the mode of contraception out of the total 2,456 clients who had contraception at the family planning clinic of UITH Ilorin, from January 2007 to December 2011.

Data collection procedure and analysis

All the qualified clients based on the inclusion criteria were identified from the family planning clinic database, their records were retrieved and relevant data extracted. Data of interest included age, parity, level of education, marital status, religion, number of children alive, source of information about implanon, side effects experienced, number that discontinued and the reasons for discontinuation. Collected data were presented as proportion.

RESULTS

During the study period, there were 2,456 clients who visited the University of Ilorin Teaching Hospital for contraception; out of which 88 opted for Implanon subdermal implant giving a prevalence of 3.6%. From Table 1, the mean age of implanon users was 33.4 years with a range of 20-49 years. Majority (42.1%) of the women who used Implanon sub-dermal implant were within the 30-34 years group. Women with two living children constituted 33% of the users; 1(1.1%) had none, 6 (6.8%) had one, 15(17.1%) had three, 28(31.8%) had four and 9(10.2%) had five or more children

Table 1: Socio-demographic characteristics of Implanon users

Variables	Distribution, n(%)
Age	
20-24	3(3.4%)
25-29	17(19.3%)
30-34	37(42.1%)
35-39	17(19.3%)
40-44	11(12.5%)
45-49	3(3.4%)
Number of children alive	
0	1(1.1%)
1	6(6.8%)
2	29(33.0%)
3	15(17.1%)
4	28(31.8%)
≥5	9(10.2%)
Level of education	
None	1(1.1%)
Primary	4(4.6%)
Secondary	20(22.7%)
Tertiary	58(65.9%)
Not stated	5(5.7%)
Religion	
Islam	41(46.6%)
Christianity	46(52.3%)
Not stated	1(1.1%)
Marital status	
Married	87(98.9%)
Single	1(1.1%)

alive. Majority 78(88.6%) of the clients had attained at least secondary education, 4(4.6%) had primary education, 1(1.1%) had no formal education while the educational status of 5(5.7%) were not stated. All the clients except one were married 87(98.9%); Christians constituted 52.3% of users and 46.6% were Muslims.

As shown in Table 2, most (81.1%) of the studied clients had information about Implanon from the Family Planning Clinic personnel; 8% had their information from friends and or relatives; 2.3% each had their information from poster and public campaigns. Only about 1% of the clients traced their source of information to Television programmes (Table 2).

Table 2: Source of information about Implanon

Source	Distribution, n(%)
Clinic personnel	72(81.8%)
Radio	7(8.0%)
Friend/relative	4(4.5%)
Public campaign	2(2.3%)
Posters	2(2.3%)
TV	1(1.1%)

From the study in Table 3, 22 of the clients representing 25% reported side effects following administration of Implanon. The commonest side effect reported was menstrual irregularity (59%), followed by weight gain and amenorrhoea (9% each). From Table 4, 23 out of 88 clients (26.1%) discontinued the Implanon during the study period. Out of this number, 35% discontinued because of desire to get pregnant, 26.1% discontinued it due to menstrual irregularity while 13% discontinued because of weight gain.

Table 3: Side effects profile of Implanon

Side effect	Distribution, n(%)
Menstrual irregularity	13(59.0%)
Weight gain	2(9.0%)
Amenorrhea	2(9.0%)
Abdominal bloating	1(4.6%)
Headache	1(4.6%)
Menstrual problem with Headache	1(4.6%)
Mood changes	1(4.6%)
Breast pain	1(4.6%)

Table 4: Reason for discontinuing Implanon

Reason	Distribution, n(%)
Desire for pregnancy	8(35.0%)
Menstrual irregularity	6(26.1%)
Weight gain	3(13.0%)
Reason not stated	2(8.7%)
Amenorrhea	1(4.3%)
Abdominal bloating	1(4.3%)
Mood changes	1(4.3%)
Bone pain	1(4.3%)

DISCUSSION

In this study, 3.6% of women using contraceptive methods in this facility used Implanon. This was higher compared to the report from the UK where 2% of contraceptive users used implanon (Monga *et al.,* 2011). Even though the reason for the observed higher value was not clear from this study, it may be due in part to the fact that the family planning personnel had direct contact with the clients who either visited the clinic at will or were referred as such were able to convince them. It is also possible for women with unmet need to readily accept the new contraceptives due to its campaigns of relatively minimal side effect with ease of usage.

Despite the fact that implanon has been found to be appropriate for teenagers (Clerk *et al.* 2006), no teenager or adolescent made use of Implanon during the study period. This showed the limited patronage of contraceptives by adolescents in this society and Africa at large. Societal norms in developing countries which invariably shuns on adolescent engagement in sexual activity and consequently neglecting the obvious widespread of unprotected sexual activity among adolescents with attendant consequences of teenage pregnancies should be addressed.

Most of the acceptors of Implanon contraception

in this study were educated with 88.6% having at least secondary education. Educated clients could be better informed about the needs for contraceptives vis-à-vis the side effects and ease of usage of the various available methods. A strong association has been reported from city slums in Kenya between women empowerment and choice of family planning as it enables them to have a say in fertility preference, use and choice of family planning methods (Okech *et al.,* 2011). Most of the acceptors of Implanon contraception were married (98.9%) further emphasizing the existence of cultural and attitudinal restriction on single women with regards to contraceptive uptake. Mekonnen *et al.,* (2011) reported from south central Ethiopia that married women with at least primary level of education were more likely to embrace contraception.

In this study, 25% of the users had side effects; this was lower than the 50% reported by Sergent *et al.,* (2004). The commonest side effect reported by users was menstrual irregularities (59%); this is similar to reports by Aisien *et al.,* (2010), who reported menstrual abnormalities as the major side effect reported by Implanon users in Benin City, Nigeria. The two commonest reasons for discontinuation of Implanon were the desire to get pregnant (35%) and menstrual disturbance (26.1%) respectively. However, most other studies reported menstrual abnormalities as the leading cause of discontinuation (Funk *et al.,* 2005, Sergent *et al.,* 2004, Lakha *et al.,* 2006). Aisien *et al.,* (2010) reported from Benin City that most of the subjects found menstrual abnormalities tolerable with adequate counseling. The lower rate of discontinuation arising from menstrual complaint in this study may be a reflection of the multiple counseling sessions before and after insertion. The continuation rates for implant use as reported by Ladipo *et al.,* (2005) were higher among those who have had adequate pre-insertion counseling; thus, improvement in counseling practiced will reduce the discontinuation rate.

The lack of post insertion complication reported in this study is in agreement with the study of Mutihir *et al.,* (2008) from Jos, Nigeria. The Pearl index found in this study was 0% as there was no pregnancy among users over the five year period. This is in agreement with reports of Affandi *et al.,* (1999), Funk *et al.,* (2005) and Power *et al.,* (2007).

CONCLUSION

Implanon is a highly effective long term reversible hormonal contraceptive, useful for different groups of women with a tolerable side effect profile. However, its benefits are being underutilized due to low uptake among contraceptive users. Public enlightenment should be vigorously pursued to improve awareness among women about Implanon usage in order to increase its uptake. In addition, wider access to Implanon should be ensured with adequate training of providers to minimize complications at insertion. The role of effective contraception should be emphasized in adolescent health program and public enlightenment to end the exclusion of this vulnerable group from contraception services.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Affandi, B., Kerver, T., Geurts, T.B. and Coelingh-Bennink, H.J. (1999). A pilot efficacy study with a single rod contraceptive implant [implanon] in 200 Indonesia women treated for greater than or equal to 4 years. *Contraception 59:167-174.*

Aisien, A.O. and Enosolease, M.E. (2010). Safety, efficacy and acceptability of implanon- a single rod implantable contraceptive (etonogestrel) in University of Benin Teaching Hospital. *Niger J Clin Pract 13:331-335.*

Association of Reproductive Health Professionals (2008). History of subdermal contraceptive implants. Association of Reproductive health bulletin.

Burkman, R.T. (2007). Contraception and family planning. In: Decherney, A.H., Nathan, L., Goodwin, T.M. and Lauren, N. (Editors). Current Diagnosis and Treatment, Obstetrics and Gynaecology. 9th edition. McGraw Hill, New York.

Clerk, N.T. and Ladipo, O.A. (2006). Contraception. In: Agboola, A. [Editor]. Textbook of Obstetrics and Gynaecology for Medical Students. 2nd edition. Heinemann Educational Books [Nig] Plc; Lagos.

Funk, S., Miller, M.M., Mishell, D.R. Jnr, Archer, D.F., Poindexter, A., Schmidt, J. and Zampaglione, E. for the Implanon US Study Group. (2005). Safety and efficacy of implanon, a single rod implantable contraceptive containing etonogestrel. *Contraception 71:319-26.*

Ladipo, O.A. and Akinso, S.A. (2005). Contraceptive implants. *Afr J Reprod Health 9:16-23.*

Lakha, F. and Glasier, F. (2006). Continuation rates of Implanon in the United Kingdom: Data from an observational study in a clinical setting. *Contraception 74:287-289.*

Mascarenhas, L. (1998). Insertion and removal of implanon. *Contraception 58(suppl):79S-83S.*

Mekonnen, W. and Worku, A. (2011). Determinants of low family planning use and high unmet need in Butajira District, South Central Ethiopia. *Reprod Health 8: 37-44.*

MerkiFed, G.S., Imthurn, B. and Seifert, B. (2008). Effects of the progestogen-only contraceptive implant, implanon on cardiovascular risk factors. *Clin Endocrinol [Oxford] 68:355-360.*

Monga, A. and Dobbs, S. (2011). Fertility control, contraception and abortion. In: Monga, A. and Dobbs, S. (Editors). Gynaecology by Ten Teachers. 19th edition. Book Power, London.

Mutihir, J.T. and Daru, P.H. (2008). Implanon subdermal implant: a 10 month review of acceptability in Jos, North central Nigeria. *Niger J Clinic Pract. 11(4):320-323.*

Okech, T.C., Wawire, N.W. and Mbwru, T.K. (2011). Contraceptive use among women of reproductive age in Kenya's slums. *Int J Bus Soc Sci; 2: 22-43.*

Okpere, E. (2007). Subdermal contraceptive implants [implanon]. In: Okpere, E. [Editor]. Clinical Gynaecology. Revised edition. Mindex Publishing Company Ltd., Benin City.

Organon [USA] Inc. (2006). Physician insert: Implanon [etonogestrel implant]. Organon [USA] Inc., Roseland.

Power, J., French, R. and Cowen, F. (2007). Subdermal implantable contraceptives versus other forms of reversible contraceptives or other implants as effective methods of preventing pregnancy. *Conchrane Database Syst Rev 18:CD001326.*

Sergent, F., Clamegeran, C., Bastard, A.M., Verspyak, E. and Marpeau, L. (2004). Acceptability of etonogestrel-containing contraceptive implant [Implanon]. *J Gynecol Obstet Biol Reprod 3:407-415.*

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ORIGINAL ARTICLE

Utilisation and diagnostic yield of large bowel endoscopy at Korle-Bu Teaching Hospital

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Large bowel endoscopy, the most accurate diagnostic investigation of the colon and rectum has been available at the Korle-Bu Teaching for close to two decades and has been used mostly for diagnosis. This retrospective study assessed patients who have undergone large bowel endoscopy, with the aim of defining the utilization of the diagnostic yield and the predominance of the varied methods. From January 1998 to December 2011, a total of 2,151 patients comprising 1,302(60.5%) males and 763(35.5%) females underwent large bowel endoscopy. Patient age ranged from 8 to 100 years with a median age of 53 years and an inter-quartile range of 25 - 72 years. The proportion of the varied methods was: colonoscopy (832; 39%), flexible sigmoidoscopy (704; 33%), rigid sigmoidoscopy (406; 19%) and proctoscopy (192; 9%). Bleeding per rectum (57.0%) was the commonest primary complaint with an overall diagnostic yield of 48.4%. In 888(41.6%) cases no pathology was found. Haemorrhoidal disease accounted for 690(32.3%) cases followed by tumours 191(9.0%). Sigmoidoscopy (both rigid and flexible) diagnosed 141(95.3%) of the tumours and colonoscopy diagnosed the remaining 7(4.7%) tumours Complete colonoscopy was achieved in 491(59%) cases scheduled for colonoscopy. In most symptomatic cases the diagnostic yield of endoscopy was high with tumours being the second commonest diagnosis after haemorrhoids. Many of the tumours were diagnosed with the sigmoidoscope. It is therefore recommended that flexible sigmoidoscopy be made available in all hospital in Ghana.
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Keywords: Rectal bleeding, Colorectal symptoms, colon, bowel tumours, Ghana

INTRODUCTION

Lower gastrointestinal endoscopy: proctoscopy, sigmoidoscopy and colonoscopy, is a standard investigative procedure performed on the anus, rectum and colon. It offers direct visualization of the mucosa of the intestine and the choice of modality to perform is guided by the patients' risk level for large bowel cancer and availability of type of endoscopy and expertise (Pignone *et al.*, 2002; Rex *et al.*, 2009).

Proctoscopy examines the anal canal and rectum; rigid sigmoidoscopy examines the rectum and distal sigmoid colon whiles the flexible sigmoidoscope examines as far as the splenic flexure of the colon. The introduction of the flexible sigmoidoscopy has witnessed a decline in the use of the rigid sigmoidoscope worldwide because of patient comfort associated with the former, higher diagnostic yield as well as the ease of carrying out a flexible sigmoidoscope (Corman, 2005). Indeed, the gold standard colonoscopy, which examines the entire large bowel, has revolutionized the management of colonic diseases due to its relatively safe and low incidence of serious complication (Nelson *et al.*, 2002).

While this invaluable service has been provided at the Korle-Bu Teaching Hospital for close to two decades, no study has been conducted to assess the utilization and diagnostic yield of the various techniques. This study was thus conducted to provide baseline data and reference for future studies in the area of lower gastro-intestinal endoscopy and better still form the basis for protocol development and

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policy formulation on endoscopy practice in hospitals in Ghana.

MATERIALS AND METHODS

A retrospective, single centre study was conducted on consecutive patients who had lower GIT endoscopy at the Korle-Bu Teaching Hospital from January 1998 to December 2011. Data source was the register at the Endoscopy unit.

For each patient, age, gender and date of endoscopy were recorded. The indications for the test were noted and in cases where more than one indication is given, these were recorded as secondary and tertiary complaints. The type of endoscopic procedure, the extent of examination achieved and the endoscopic diagnosis were also recorded.

For patients in whom a tumour was identified at sigmoidoscopy, complete bowel examination was achieved with colonoscopy and/or barium enema. In view of the fact that the primary examination the patients were billed to undergo was sigmoidoscopy, such cases are captured under sigmoidoscopy in this study.

All procedures were performed by general surgeons and gastroenterologists, who are the endoscopists, after they have thoroughly reviewed the case. The patients comprised of those referred from hospitals and clinics within the Greater Accra metropolis as well as those receiving care in Korle-Bu Teaching Hospital.

Statistical analysis

The data extracted was entered into excel spread sheet and later transferred to IMB SPSS version 19, New York, for statistical analysis. Chi square test was done and p value < than 0.5 for considered significant.

RESULTS

A total of 2,151 patients comprising 1,302(60.5%) males and 763(35.5%) females, with ages ranging from 8 to 100 years and a median age of 53 years (IQ range: 25 - 72 years) underwent lower gastrointestinal endoscopy within the study period under review. Varied proportions of data for the variables studied were available,as shown in Table 1. . For all age groups and the various types of endoscopy males pre-dominated females (Figure 1 and 2).

Table 1: Proportions of data available on variables studied

Variable	No. of available data	Percentage available data
Age	1950	90.7%
Sex	2065	96.4%
Date of examination	2151	100.0%
Primary Complaint	1941	90.2%
Endoscopic Diagnosis	2137	99.3%
Type of endososcopic technique	2134	99.2%
Location of Lesion	2096	97.4%

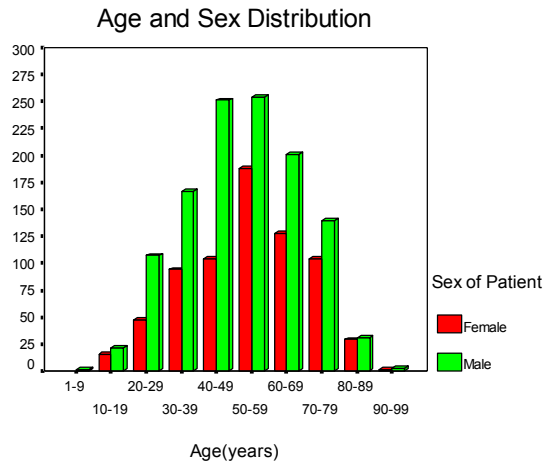

Figure 1: Age and sex distribution of the cases

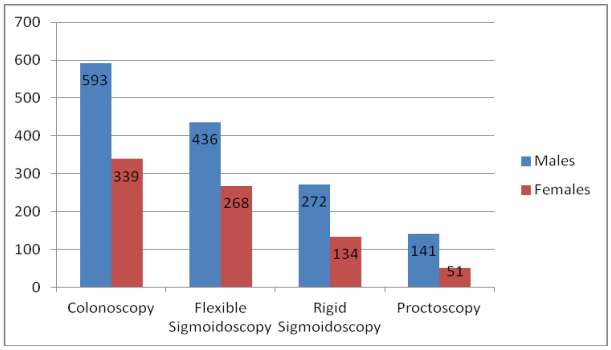

Figure 2: Distribution of the various endoscopic types by sex

There was a progressive increase in the utilization of endoscopic evaluation of the large bowel over the study period. It is worth noting that there was a drastic decline in 2007 (Table 2). The types of endoscopy performed were: colonoscopy 832(39%), flexible sigmoidoscopy 704(33%), rigid sigmoidoscopy 406(19%) and proctoscopy 192(9%) (Table 2). Before the age of 50 years usage of flexible sigmoidoscopy pre-dominate marginally followed by colonoscopy, rigid sigmoidoscopy and proctoscopy. However, after the age of 50 years usage of colonoscopy substantially pre-dominate followed by flexible sigmoidoscopy, rigid sigmoidoscopy and proctoscopy (Figure 3).

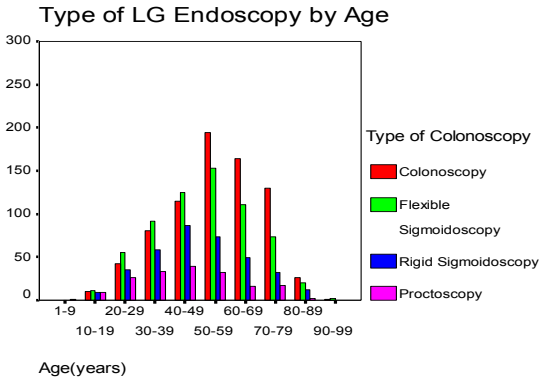

Figure 3: Types of Lower gastrointestinal endoscopy by age

Table 2: Annual frequencies of the various type of lower gastrointestinal Endoscopy

Years	Lower GI Endoscopy	Colonoscopy	Flexible Sigmoidoscopy	Rigid Sigmoidoscopy	Proctoscopy
n	*2151*	*832*	*704*	*406*	*192*
1998	39(1.8%)	15(1.8%)	7(1.0%)	16(3.9%)	0(0.0%)
1999	46(2.1%)	29(3.5%)	8(1.1%)	9(2.2%)	1(0.5%)
2000	46(2.1%)	23(2.8%)	17(2.4%)	3(0.7%)	1(0.5%)
2001	65(3.0%)	33(4.0%)	31(4.4%)	0(0.0%)	1(0.5%)
2002	95(4.4%)	40(4.8%)	48(6.8%)	3(0.7%)	6(3.1%)
2003	116(5.4%)	42(5.0%)	49(7.0%)	21(5.2%)	5(2.6%)
2004	120(5.6%)	52(6.3%)	26(3.7%)	33(8.1%)	6(3.1%)
2005	165(7.7%)	66(7.9%)	25(3.6%)	38(9.4%)	23(12.0%)
2006	190(8.8%)	99(11.9%)	43(6.1%)	39(9.6%)	9(4.7%)
2007	89(4.1%)	20(2.4%)	13(1.8%)	37(9.1%)	17(8.9%)
2008	404(18.8%)	215(25.8%)	166(23.6%)	5(1.2%)	15(7.8%)
2009	247(11.5%)	23(2.8%)	111(15.8%)	155(38.2%)	56(29.2%)
2010	246(11.4%)	106(12.7%)	109(15.5%)	19(4.7%)	9(4.7%)
2011	283(13.2%)	69(8.3%)	151(21.4%)	28(6.9%)	43(22.4%)

Bleeding per rectum (57.0%) was the commonest primary complaint. Other primary indications for lower GI endoscopy are as shown in the Table 3. When the studied population was stratified based on gender, significantly higher proportion of the males presented with bleeding per rectum (61.3%), haemorrhoids (1.8%) and anal discharge (1.2%) as compared to their female counterparts (49.3%, $p < 0.0001$; 0.7%, $p = 0.0454$ and 0.3%, $p = 0.0375$ for bleeding per rectum, haemorrhoids and anal discharge respectively). However, abdominal pain, follow-up post-surgery, abdominal mass as well as follow-up for IBD were more significantly associated with the female as compared to the male (Table 3). Multiple complaints were noted in 263(12.2%) patients who had two complaints and 6(0.3%) patients who had 3 complaints.

No pathology was found in a large number of patients 888(41.6%). Haemorrhoidal disease was the commonest pathology identified, 690(32.3%), followed by tumours 191(9.0%). More than one pathology was identified in 97(4.5%) patients. Other diseases found are detailed in Table 4. Sigmoidoscopy (both rigid and flexible) diagnosed 141 (95.3%) of the tumours and 44(57.9%) of the

Table 3: Frequencies of the primary complaints stratified by gender

Primary Complaint	Total	Female	Male	P value
n	*1941*	*696*	*1245*	
Bleeding per rectum	1106(57.0%)	343(49.3%)	763(61.3%)	< 0.0001
Abdominal Pain	136(7.0%)	69(9.9%)	67(5.4%)	0.0002
Diarrhoea	137(7.1%)	54(7.8%)	83(6.7%)	0.3677
Follow-up post-surgery	67(3.5%)	45(6.5%)	22(1.8%)	< 0.0001
Constipation	69(3.6%)	27(3.9%)	42(3.4%)	0.5638
Anorectal Pain	58(3.0%)	23(3.3%)	35(2.8%)	0.5404
Anaemia	51(2.6%)	18(2.6%)	33(2.7%)	0.9322
Change in bowel habit	64(3.3%)	23(3.3%)	41(3.3%)	0.9892
Rectal Mass	48(2.5%)	22(3.2%)	26(2.1%)	0.1445
Abdominal Mass	38(2.0%)	21(3.0%)	17(1.4%)	0.0118
Haemorrhoids	28(1.4%)	5(0.7%)	23(1.8%)	0.0454
Follow-up for IBD	23(1.2%)	14(2.0%)	9(0.7%)	0.0119
Screening	20(1.0%)	7(1.0%)	13(1.0%)	0.9359
Fistula-in-ano	16(0.8%)	3(0.4%)	13(1.0%)	0.1519
Anal Discharge	17(0.9%)	2(0.3%)	15(1.2%)	0.0375
Weight loss	19(1.0%)	9(1.3%)	10(0.8%)	0.2931
Abnormal Barium Enema	11(0.6%)	4(0.6%)	7(0.6%)	0.9720
Follow-up after polypectomy	13(0.7%)	2(0.3%)	11(0.9%)	0.1225
Flatulent dyspepsia	4(0.2%)	0(0.0%)	4(0.3%)	0.1344
Perianal Ulcer	3(0.2%)	0(0.0%)	3(0.2%)	0.1950
Pruritus Ani	3(0.2%)	1(0.1%)	2(0.2%)	0.9273
Others	10(0.5%)	4(0.6%)	6(0.5%)	0.7842

P values were generated from chi-square analysis comparing the male and female

Table 4: Endoscopic Diagnosis of the studied population (*n = 2134*)

Primary Findings	Prevalence
Normal Findings	888(41.6%)
Haemorrhoids	690(32.3%)
Tumours	191(9.0%)
Proctocolitis	90(4.2%)
Polyp	76(3.6%)
Diverticular Disease	65(3.0%)
Anal Fissure	30(1.4%)
Fistula-in-ano	18(0.8%)
Stenosis in the colon	16(0.7%)
Upper Gastrointestinal Bleed	12(0.6%)
Pus	9(0.4%)
Pale Mucosa	8(0.4%)
Ulcerative Colitis	10(0.5%)
Others	31(1.5%)

polyps while the rest of these lesions were diagnosed with the colonoscope 7(4.7%) of the tumours and 32(42.1%) of the polyps. Caecal intubation (complete colonoscopy) was achieved in 491 (59.0%) cases that were planned to have colonoscopy.

DISCUSSION

Endoscopy has revolutionized the management of diseases of the large bowel not only because it has become the diagnostic method of choice but also a major therapeutic modality for some pathologies of the bowel (Wolff and Shinya, 1971). Consistent diagnostic endoscopic service has been available at the Korle-Bu Teaching hospital since 1995, however, trends of its utilization and diagnostic yield for large bowel symptoms has not been studied, hence, the need for this study. This study reviewed cases from 1998 because data from 1995 to 1997 were unavailable.

Being a retrospective study the inherent problem of completeness of data was encountered. Data was hand-recorded and in some cases were not legible. Despite these, the completeness of the available data of interest ranged between 90 and 100 percent which is adequate for a meaningful analysis.

There was a high utilization of Colonoscopy and flexible sigmoidoscopy in this study. This is the result of both recognition of these procedures as gold standard for evaluating and treating lesions in the colon and rectum (Cappell and Friedel, 2002) and the availability of skilled personnel carrying out these procedures in the hospital. These personnel are, however, not dedicated endoscopists contributing to the low rate of complete colonoscopy (59%). Ten general surgeons and four gastroenterologists acting as the endoscopists take turns each in the course of the week to perform the procedure. The time allotment is small for each person who performs both gastroscopies and the lower gastrointestinal endoscopies at the same sitting.

Increase use of flexible sigmoidoscopy was witnessed with a decreasing utilization of rigid sigmoidoscopy over the studied period. In 2008, the later was the least performed endoscopic procedure. This follows the worldwide trend because of the superiority of the flexible sigmoidoscpe in detecting lesions and the ease of performing procedures such as biopsy and polypectomy with it (Traul *et al.*, 1983). In a retrospective study by Rao *et al.,* 33.9% of patients who were declared normal by rigid sigmoidoscopy had lesions on flexible sigmoidoscopy (Rao *et al.*, 2005).

Selection of a type of endoscopic procedure that was performed was informed by the patients' presenting symptoms and the likelihood of identifying a neoplasm which explains the predominance of flexible sigmoidoscopy over colonoscopy in patients who were aged less than 50 years, and colonoscopy exceeding flexible sigmoidoscopy in cases who were 50 years and older. The overall diagnostic yield in this study was 48.4%.

Detection of tumours in the large bowel is the single most important reason for endoscopy either in symptomatic patients or for screening purposes (Doubeni *et al.*, 2013). In a large study involving 16,433 symptomatic cases who underwent flexible sigmoidoscopy over a 16 year period in a single colorectal unit in south of England, it was shown that the chance of missing a proximal lesion with this procedure is about 2.5% (Thompson *et al.*, 2008). In West Africa about 50% of colorectal cancers are located in the rectum of which 78% are within reach of the examining finger, with another 20% in the sigmoid, descending and splenic flexure of the colon (Dakubo *et al.*, 2010; Irabor and Adedeji, 2009; Naaeder and Archampong, 1994).

This large proportion of tumours are recognisable with the flexible sigmoidoscope hence together with its short learning curve and ease of performance, it is appropriate to recommend its wide availability in all district hospitals (where it is currently unavailable) in the country and the resident doctors taught to use it. This has the potential of increasing the detection rate of early cancers since many cases of colorectal cancer that present late to the tertiary centres will be identified early at the peripheral hospitals and then referred. About 95% of the tumours and 58% of the polyps in this study were diagnosed with the sigmoidoscope (both rigid and flexible) while about 5% of the tumours and 42% of the polyps were diagnosed with the colonoscope from this study. Right colon cancers account for about 30% of colorectal cancer(Dakubo *et al.*, 2010). The observed low rate of right colon tumours noted in this study could be as a result of the stage and presentation of these tumours. They are mostly operated upon without endoscopy because they present as large obstructing lesions in emergent states.

In this study, proctosopy has been included as an endoscopic procedure for completeness of the data because it is routinely performed at the endoscopy unit. Its usage is limited to evaluation of the anal canal and lower rectum and permits biopsy of lesions as well as aiding in sclerotherapy of haemorrhoids. In young patients presenting with bright red bleeding and anal symptoms suggestive of haemor-

rhoids (feeling of warmth in the anus, pruritus ani, anal discharge and protruding anal mass at defaecation) this was the only test done since the likelihood that a proximal tumour will be missed is very low (Vening *et al.*, 2010). Additionally, although during sigmoidoscopy the anal canal can be visualized, it is not an effective substitute for proctoscopy.

Bleeding PR, abdominal pain, diarrhoea and constipation were the most frequent reasons for which patients underwent large bowel endoscopic procedure. These indications agree fairly well with those of the Clinical Category of European Panel on The Appropriatness of Gastrointestinal Endoscopy II (EPAGE II) (Juillerat *et al.*, 2009). This study also found that other relatively frequent indications as defined in the EPAGE II Clinical categorization guideline were pertinent and included iron deficiency anaemia, follow-up post colectomy, follow-up for IBD and screening for CRC. However, appropriateness analysis based on EPAGE II was not part of the scope of this study.

In more than one third of the patients, the colorectal mucosa and anal epithelium were deemed normal. However, it is important to note that colonoscopy was intended in only 39% of the cases. Even in this group completeness was achieved in only in 59%. These may contribute to a low diagnosis rate even though patients in whom colonoscopy was incomplete had their large bowel evaluated by double contrast barium enema, the data of which was not available for this study. Haemorrhoidal disease is not uncommon in the tropics as was thought in the past. It was the commonest disease reported in 32,3% of the patients signifying that other predisposing factors to the development of haemorrhoidal disease beyond diet are at play. Other common diseases noted were tumours (9.0%), proctocolitis (4.2%), polyps (3.6%) and diverticular disease (3.0%) cases. This compares disproportionately with a European series of lower Gastrointestinal endoscopy done over an 11 year period involving 11,550 cases in which cancers accounted for 4-6%, Inflammation, 9-15%, polyps 9-16% and diverticular disease 21-37% (Loffeld and van der Putten, 2005). Diverticular disease was found in 3% of patients which is understandably lower than the 4.5% reported from this hospital in patients who presented with haematochezia (Dakubo *et al.*, 2008). Bleeding is the commonest form in which diverticular disease presents in Tropical Africa and thus explaining the earlier higher frequency of this disease (Archampong *et al.*, 1978; Baako, 2001). Notably a very rare condition in blacks in the Tropics 10 cases of ulcerative colitis were diagnosed.

In patients that colonoscopy was planned, caecal intubation was achieved in 59%. This is higher than the 30.4% complete colonoscopy reported earlier when a smaller proportion (181 patients) of this population was studied (Dakubo *et al.*, 2008). Reasons for the low complete colonoscopy rate could be due to redundancy of the colon: sigmoid and transverse colon, difficulty in achieving adequate bowel preparation due to the bulky nature of stools, and the time available for each endoscopist who performs both upper and lower gastrointestinal endoscopies at the same sitting (Kim *et al.*, 2000). This low complete colonoscopy rate falls far short of the 92% reported from other centre were the procedures are performed by dedicated endoscopists (Selehi *et al.*, 2008). It is worrying when viewed against the background that 16.2% and 7.2% of tumours are located in the caecum and ascending colon respectively in our experience (Juillerat *et al.*, 2009).

CONCLUSION

Lower gastrointestinal endoscopy is an invaluable investigative procedure and in symptomatic patients has a high diagnostic yield. Most of the tumours were diagnosed with the flexible sigmoidoscope. It is recommended that flexible sigmoidoscopy be made widely available in all health institutions in Ghana.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Archampong EQ, Christian F, Badoe EA (1978). Diverticular disease in an indigenous African

community. *Annals of the Royal College of Surgeons of England* 60(6): 464-470.

Baako BN (2001). Diverticular disease of the colon in Accra, Ghana. *The British journal of surgery* 88 (12): 1595.

Cappell MS, Friedel D (2002). The role of sigmoidoscopy and colonoscopy in the diagnosis and management of lower gastrointestinal disorders: endoscopic findings, therapy, and complications. *The Medical clinics of North America* 86(6): 1253-1288.

Corman ML (2005). *Colon And Rectal Surgery*. 5 edn. Lippincott Williams & Wilkins: Philadelphia.

Dakubo J, Kumoji R, Naaeder S, Clegg-Lamptey J (2008). Endoscopic evaluation of the colorectum in patients presenting with haematochezia at korle-bu teaching hospital accra. *Ghana medical journal* 42(1): 33-37.

Dakubo JC, Naaeder SB, Tettey Y, Gyasi RK (2010). Colorectal carcinoma: an update of current trends in Accra. *West African journal of medicine* 29(3): 178-183.

Doubeni CA, Weinmann S, Adams K, Kamineni A, Buist DS, Ash AS*, et al.* (2013). Screening colonoscopy and risk for incident late-stage colorectal cancer diagnosis in average-risk adults: a nested case-control study. *Annals of internal medicine* 158(5 Pt 1): 312-320.

Irabor D, Adedeji OA (2009). Colorectal cancer in Nigeria: 40 years on. A review. *European journal of cancer care* 18(2): 110-115.

Juillerat P, Peytremann-Bridevaux I, Vader JP, Arditi C, Schussele Filliettaz S, Dubois RW*, et al.* (2009). Appropriateness of colonoscopy in Europe (EPAGE II). Presentation of methodology, general results, and analysis of complications. *Endoscopy* 41(3): 240-246.

Kim WH, Cho YJ, Park JY, Min PK, Kang JK, Park IS (2000). Factors affecting insertion time and patient discomfort during colonoscopy. *Gastrointestinal endoscopy* 52(5): 600-605.

Loffeld RJ, van der Putten AB (2005). The annual yield of diagnostic endoscopy of the lower digestive tract. *European journal of internal medicine* 16(1): 37-40.

Naaeder SB, Archampong EQ (1994). Cancer of the colon and rectum in Ghana: a 5-year prospective study. *The British journal of surgery* 81 (3): 456-459.

Nelson DB, McQuaid KR, Bond JH, Lieberman DA, Weiss DG, Johnston TK (2002). Procedural success and complications of large-scale screening colonoscopy. *Gastrointestinal endoscopy* 55(3): 307-314.

Pignone M, Rich M, Teutsch SM, Berg AO, Lohr KN (2002). Screening for colorectal cancer in adults at average risk: a summary of the evidence for the U.S. Preventive Services Task Force. *Annals of internal medicine* 137(2): 132-141.

Rao VS, Ahmad N, Al-Mukhtar A, Stojkovic S, Moore PJ, Ahmad SM (2005). Comparison of rigid vs flexible sigmoidoscopy in detection of significant anorectal lesions. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 7(1): 61-64.

Rex DK, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM (2009). American College of Gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. *The American journal of gastroenterology* 104(3): 739-750.

Selehi S, Leung E, Wong L (2008). Factors affecting outcomes in colonoscopy. *Gastroenterology nursing : the official journal of the Society of Gastroenterology Nurses and Associates* 31(1): 56-63.

Thompson MR, Flashman KG, Wooldrage K, Rogers PA, Senapati A, O'Leary DP*, et al.* (2008). Flexible sigmoidoscopy and whole colonic imaging in the diagnosis of cancer in patients with colorectal symptoms. *The British journal of surgery* 95(9): 1140-1146.

Traul DG, Davis CB, Pollock JC, Scudamore HH (1983). Flexible fiberoptic sigmoidoscopy--the Monroe Clinic experience. A prospective study of 5000 examinations. *Diseases of the*

colon and rectum 26(3): 161-166.

Vening W, Willigendael EM, Tjeertes EK, Hulsewe KW, Hoofwijk AG (2010). Timing and necessity of a flexible sigmoidoscopy in patients with symptoms suggestive of haemorrhoids. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 12(2): 109-113.

Wolff WI, Shinya H (1971). Colonofiberoscopy. *JAMA : the journal of the American Medical Association* 217(11): 1509-1512.

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ORIGINAL ARTICLE

Stigma and discrimination associated with HIV/AIDS in health care settings: a comparative study in two hospitals of different categories in Douala-Cameroon

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The response to the human immunodeficiency virus epidemic faces many challenges with stigma and discrimination being two of them. The aim of this study is to determine the extent of effects of stigmatization and discrimination against people living with HIV-AIDS, and the influence of the type of hospital structure, in the manifestations of stigma and discrimination. A prospective cross sectional study was conducted among a total of 400 patients, using a pre-tested questionnaire. An observation form was also filled to evaluate attitudes and behaviour of health care providers towards patients. Chi-Square test and Fisher test were used to test association between two variables, then multi logistic regression tests were done to check predictive factors of discrimination. The level of significance was chosen at p<0.05. Among the participants, 104 (26%) patients reported having been victims of discrimination. Laquintinie hospital of Douala has a risk factor for blames and maltreatment (p = 0.0060) and (p= 0.0091) respectively. Also 152 (76.1%) patients of Laquintinie vs 103 (51.5%) of Nylon have been victims of stigmatization. The stigmatizing elements were: the name of the treatment center (p<0.0001) and the unconfidential manner of handling medical files (p=0.0527). Among the 400 patients, fifty nine (14.8%) avoided going to the hospital because of past experience of stigma and discrimination. Patients encounter several difficulties and those related to stigma and discrimination experienced in a hospital milieu can particularly constitute obstacles to better health seeking and therapeutic adherence. The human immunodeficiency virus infection response strategy should address stigma and discrimination by reviewing the management of treatment centers, elaborating relevant public health policies and training of healthcare practitioner.

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Keywords: HIV/AIDS; Stigma; Discrimination; Laquintinie; Nylon; Douala

INTRODUCTION

The response to the human immunodeficiency virus (HIV) epidemic is gradually improving as a result of universal access to antiretroviral (ARV) (Oku *et al.,* 2013). The quality and life expectancy of people infected has extended significantly, although many challenges remain, notably that of stigma and discrimination (S & D) (Vanden Driessche *et al.,* 2009).

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The Joint United Nations Programme on HIV/AIDS (UNAIDS) defines stigma as a "process of devaluation of people either living with or associated with HIV and AIDS". Discrimination follows stigma and is the unfair and unjust treatment of an individual based on his or her real or perceived HIV status" (Vanden Driessche *et al.,* 2009). AIDS-related S & D refers to prejudice, negative attitudes, abuse and maltreatment directed at people living with HIV and AIDS (Lifson *et al.,* 2013).

Since the beginning of the epidemic, S & D have been identified as the main obstacles in the way of

effective responses to HIV (Mashimo *et al.,* 2001). S & D associated with HIV is a complex social process which interacts with and reinforces existing S & D associated with gender, race and poverty (Vanden Driessche *et al.,* 2009). Numerous studies have documented attitudes of healthcare providers toward people living with HIV (PLHIV) (Chan *et al.,* 2008, Kremer *et al.,* 2006, Oku *et al.,* 2013, Vanden Driessche *et al.,* 2009). Although the literature characterizes the attitudes and behaviour of healthcare providers as positive and respectful, many studies also report poor communication between patients and healthcare providers (Chan *et al.,* 2008, Kremer *et al.,* 2006), which functions as a major barrier in providing proper care for these patients (Tawfik and Kinoti, 2001).

Efforts to reduce S & D associated with HIV/AIDS will not only help countries achieving the key targets for universal access and Millennium Development Goal 6, they will also protect and promote human rights, promote respect for PLHIV and other interested groups and reduce HIV transmission. Reducing stigma and discrimination related to HIV/AIDS among health care providers will be useful not only for marginalized PLHIV and their partners, but also for health professional groups themselves, whose activities will be facilitated through easier collaboration with patients. Studies indicate that health care providers delay access to health care services in an attitude of S & D (Pruss-Ustun *et al.,* 2005, Oyeyemi *et al.,* 2006).

Stigma associated with HIV in Africa has been documented to be a barrier to disclosure of HIV status (Lifson *et al.,* 2013). Attitudes involved are shame, blame and judgment among others. In a study conducted in Kenya, 43% of the study population recognizes this obstacle (Feyissa *et al.,* 2012). A recent study in Cameroon showed that, 23% of victims have lost their jobs because of S & D (Yang *et al.,* 2007). Another study conducted in Buea in Cameroon, which is one of the first in a hospital setting, showed that the major problems faced by the PLHIV with regard to S & D were gossiping and verbal abuse through insults and derogatory language. This was felt by about half of the interviewees (Nguyen *et al.,* 2009). The aim of this study is to determine the extent of effects of stigmatization and discrimination against people living with HIV-AIDS and the influence of the type of hospital structure in the manifestations of stigma and discrimination.

MATERIALS AND METHODS

Study design and context

This cross-sectional and prospective study was conducted from January to April 2013. With the advent of HIV infection in Cameroon in the early 2000s, the government organized a response to the pandemic, with the establishment of centers to care for those infected. Authorized treatment centers (ATC) of Central Hospital of Yaoundé and Douala Laquintinie hospital were thus created. These centers are mainly characterized by their geographical location and their specificity marked by isolation and orientation reserved for the reception of patients infected with HIV. Over the years, several other centers called units of care have emerged particularly in district hospitals. Unlike older support units, these new centers have no specific geographical location and are actually virtual structures. Thus, support for PLHIV is provided by structures that do not have the same configurations. One would wonder if either of these configurations would not facilitate the perception of S & D.

Study sites

The study was conducted in two antiretroviral treatment (ART) centers in Douala: the Day Care Hospital (DCH) of Laquintinie hospital (site 1) and the Health Care Unit of Nylon district hospital (HCU) (site 2). Laquintinie hospital and Nylon district hospital are the two first centers of support for PLHIV in Douala, with over 40% of all patients being treated in this city (GTRL, 2012).

Laquintinie hospital is a second category center according to health care classification in Cameroon. It was established in April 2001 with the main objective of taking care of patients with chronic diseases such as HIV/AIDS, diabetes and hypertension but this is currently, not the case. DCH takes care of only HIV positive patients and currently has

a regular line of about 4200 patients per month (GTRL., 2012). The DCH is an ATC with a complete and separately dedicated physical infrastructure and located in a barrier within the confines of Laquintinie hospital. The HCU has been in operation since 2007 with an estimated active line of 4010 patients per month, who are regularly followed up (GTRL., 2012). HCU is a virtual structure with other conditions and people living with HIV being given support.

Sampling and sample size

HIV positive subjects who came for consultation or follow up visits were consecutively integrated in the study if they were 18 years or more, and had been followed up for at least 6 months at the center. In all, 410 patients were recruited and after excluding illegible and poorly filled questionnaires, 400 (200 for each hospital) questionnaires were retained for the study. All the patients accepted to take part in the study by signing an informed consent form.

Data collection

Data were collected in the DCH of Laquintinie and the HCU of Nylon district hospital using a questionnaire addressed to the patient and the information recorded accordingly. The questionnaire was pretested on 20 patients beforehand in both hospitals. Errors were corrected and questions were finally remodeled.

The questionnaire took into consideration different variables including: Socio demographic data (sex, age, marital status, religion, ethnic group, occupation, level of education, distance of residence), duration of follow-up, ARV medication status, opinion on structural organization of treatment center (name, location of treatment center), opinion on quality of health care given (habits during care administration in Consultation rooms, Laboratory, Pharmacy, Waiting room, counseling room), opinion on policies of treatment center (frightful/death-oriented posters, anti-discrimination office), effect of S & D on health-seeking behaviour (thought of avoiding hospital, effectively avoiding hospital, and hiding of status from healthcare providers). Each questionnaire was completed within an average time of 12 – 15 minutes. A form prepared to allow investigators to learn about the attitudes of healthcare providers towards PLHIV, including nursing, switching patients to different levels of health care, on the nature of the health care organization of the unit, on measures related stigma in hospital.

Ethical considerations

The confidentiality of data collected was conserved as the information was recorded in an anonymous coded questionnaire that is decodable only by the investigator. An informed consent form was given to each patient who read after thorough explanation before the interview and signed at the end of the interview. Permission to conduct the study was obtained from the National Ethics Committee.

Data analysis

Data was recorded using the software program EXCEL 2013, then exported and analyzed using the STATVIEW version 5.0 SAS Institute (Elford et al., 2012). For the analysis, the rules of descriptive statistics for the calculation of means and proportions of different variables were used. Data was first examined to check the distribution of all different variables, and then bivariate associations between different variables were checked using the Chi-square test and the Fisher exact test. Logistic regression was done to check predictive factors of discrimination.

RESULTS

Of the 400 respondents in the study, 282(70.5%) were female while 118(29.5%) were male. The mean age of the study population was 26 ± 5 years and a modal age range 29-39 years. Majority of respondents 267(66.7%) have had secondary education. Forty-seven percent (n = 188) of the patients were Catholics, 227(65.7%) were from the local languages Bamileke and Bamoun. Also 165(41.3%) of the patients were single, while 176(44%) were married or cohabiting with their partners. There were 374(93.5%) patients on ARV drugs with 278 (69.5%) who were followed for over 2 years and only 50(14%) who were followed for less than 1

year. Most of the respondents 226(56.5%) belonged to the informal private sector.

Manifestations of Discrimination

Discrimination was estimated to be 104 (26%) for the study with the prevalence in DCH of Laquintinie being evaluated at 34.5% which is almost twice that in HCU of Nylon (17.5%). According to these results, blames emerged to be the principal manifestation with 11% prevalence (p=0.0088) (Table 1).

Prediction of risk factors of Discrimination

There was a strong association between DCH of Laquintinie, the maltreatment (OR: 5.461; 95% CI: 1.526—19.545; p=0.0091), and blames (OR: 2.631; 95% CI: 1.319-5.249; p=0.0060). There was no association between DCH, insults and poor quality services. Nylon hospital as well as age, gender and level of education were not associated with any element of discrimination (Tables 2 and 3).

Table 1: Distribution of participants according to manifestations of discrimination

Variable	DCH, n(%)	HCU, n(%)	P value
Blames	33(16.5%)	13(6.5%)	0.0088
Insults	5(2.5%)	2(1.0%)	0.5275
Maltreatment	15(7.5%)	3(1.5%)	0.0134
Poor quality services	16(8.0%)	17(8.5%)	0.4630

DCH=Day Care Hospital and HCU=Health Care Unit

Influence of structural management of hospital, behavior of health care providers and policies of treatment center on stigma

These results shows that objective findings on stigmatizing attitudes and discriminatory habits of personnel, distinctive signs were always found on medical files, occasional voluntary/Involuntary disclosure of HIV status and rare situations of blame were observed in DCH of Laquintinie but none of these were observed in HCU of Nylon. Two patients often entered the consultation room at a time in Laquintinie but in Nylon only one was always observed. In Laquintinie, more than 2 patients were received in the pharmacy often. No death-oriented poster was found. (Table 4).

Responses of participants on influence of structural organization of hospital, quality of care and policies of treatment center on stigma

From these results, 152 participants (76.1%) in DCH of Laquintinie and 103 participants (51.5%) in HCU of Nylon experienced stigmatization. Twenty eight (7.02%) participants were uncomfort-

Table 2: Predictors of discrimination in relation with blames and maltreatment

Variable	Odds Ratio	95% CI	P value
Maltreatment			
Constant= yes	0.000	0.000—1.000	0.9948
Age	1.306	0.980—1.096	0.2074
Hospital=laquintinie	5.461	1.526—19.545	0.0091
Sex=male	0.551	0.172—1.760	0.3142
Primary education	684954.351	0.000—1.000	0.9966
Secondary education	4126923.694	0.000—1.000	0.9961
Tertiary education	1313663.24	0.000—1.000	0.9964
Blames			
Constant= yes	0.092	0.005—1.723	0.1105
Age	1.017	0.981—1.053	0.3687
Hospital=laquintinie	2.631	1.319—5.249	0.006
Sex=male	0.900	0.427—1.896	0.7814
Primary education	0.385	0.031—4.844	0.4603
Secondary education	0.404	0.034—4.758	0.4713
Tertiary education	0.568	0.043—7.462	0.6668

Table 3: Predictors of discrimination in relation with insults and poor services

Variable	Odds Ratio	95% CI	P value
Poor service			
Constant= yes	0.000	0.000—1.000	0.9955
Age	1.003	0.962—1.046	0.8758
Hospital=Laquintinie	1.293	0.618—2.207	0.4951
Sex=male	0.537	0.172—2.826	0.6235
Primary education	1727047.183	0.000—1.000	0.9963
Secondary education	3253724.909	0.000—1.000	0.9962
Tertiary education	5440087.243	0.000—1.000	0.9961
Insults			
Constant= yes	0.000	0.000—1.000	0.9956
Age	0.964	0.874 —1.062	0.4572
Hospital =Laquintinie	3.084	0.566—16.815	0.1930
Sex=male	0.471	0.050—4.458	0.5117
Primary education	1096479.046	0.000—1.000	0.9963
Secondary education	688786.253	0.000—1.000	0.9965
Tertiary education	777249.354	0.000—1.000	0.9964

Table 4: Findings observed in stigmatizing and discriminatory practices of staff attitudes in the waiting room, consultation room and pharmacy

Variable	Day Care Hospital		Health Care Unit	
	Response	Frequency	Response	Frequency
Waiting room				
Nature	Reserved only for PLWHA	always	Reserved for all patients	always
Nurses to patient ratio	3/55	often	3/5	often
Distinctive signs on file	Yes	always	No	always
Involuntary/voluntary disclosure	Yes	sometimes	No	always
Blames	Yes	Very rarely	No	always
Consultation room				
Nature	Reserved only for PLWHA	always	Reserved for all patients	always
Number of patients	2	often	1	always
Presence of other personnel	1	rarely	0	always
Inward/outward movements	Yes	Yes	sometimes	rarely
Pharmacy				
Nature	Reserved only for PLWHA	always	Reserved for all patients	always
Number of patients	5	often	2	rarely
Blames	Yes	Very rarely	No	always
Inward/outward movements	Yes	rarely	Yes	Very rarely
Refusal of ARV drugs	No	always	No	always

able about the name of treatment center (p<0.0001); 44(11 %) were uncomfortable about its location; 10 (2.5%), 147(36.8%) and 17(4.25%) respectively complained of Medical files indiscretion, ARV dispensation indiscretion, indiscretion in collection of laboratory results. Seven (1.75%) participants complained of frightful/death oriented posters (p=0.0076) (Table 5).

Table 5: Influence of structural organization of hospital, quality of care and policies of treatment center on stigma

Variables	DCH, n(%)	HCU, n(%)	P value
Uncomfortable by name of center	28(14.1%)	0(0.0%)	<0.0001
Uncomfortable by location of center	25(12.5%)	19(9.5%)	0.3377
Indiscretion of medical file	8(4.0%)	2(1.0%)	0.0527
Indiscretion of ARV dispensation	77(38.5%)	70(36.0%)	0.3087
Indiscretion in collecting lab results	7(3.5%)	10(5.0%)	0.4700
Frightful/death oriented posters	7(3.5%)	0(0.0%)	0.0076

About the knowledge on an organ fighting discrimination, these results show that, 44 out of 400 interviewees (11%) indicated knowing a complaint office in case of discrimination, 16(36%) reported it to be the ward in charge's office, 9(21%) reported it to be the director's office and 10(25%) still reported it to be a complaint/suggestion box.

Effects of Stigma and discrimination on health-seeking behaviour of participants

A total of 59 (14.8%) participants avoided going to the hospital because of previous experience of S & D, and16 (4%) of participants decided to hide their HIV status in situations of seeking health care services, all of the latter being those of DCH of Laquintinie with none in HCU of Nylon (Figure 1).

DISCUSSION

The proportions of epidemiological repartition of this study are correlated with current trends of HIV

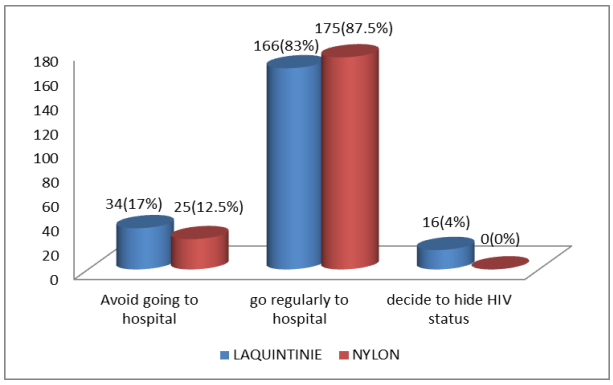

Figure 1: Health-related consequences of stigma and Discrimination

infection in Cameroon (GTRL, 2012). According to the report of the National Committee for the fight against AIDS in 2011, women were more infected than men with the most affected age group being 30-39 years with a prevalence rate of 8.1%. Globally, the prevalence of discrimination was estimated to be 26% with that of DCH of Laquintinie hospital estimated at 34.5% and HCU of Nylon at 17.5%. These two values are lower compared to the 36% revealed by Andrianasolo *et al.,* (2011), in Madagascar. Similarly, Peretti-Watel *et al.,* (2007), showed that 26.28% patients experienced discrimination by medical personnel. Elford, (2012), demonstrated that half of all cases of HIV discrimination were in health care settings. The results of this study revealed that the major manifestations were: blame, insults, maltreatment, and poor health services.

The most prominent manifestation of S & D was blame which was estimated to be 16.5%. This is significantly greater than estimates observed in DCH of Laquintinie (11.5%) and HCU of Nylon (6.5%). The results of this study confirmed that there were very rare cases of blame in Laquintinie with none in Nylon. Occurrence in DCH could be explained by the workload. Only three nurses provide care services for many patients who present themselves every day. This is in line with reports of Adebajo *et al.,* (2003) and Letamo, (2005). The results showed that the abuse experienced by PLHIV to DCH of Laquintinie (7.5%) were much higher than those experienced in HCU of Nylon (1.5%).

Maltreatment which can be manifested in this case as deliberately wasting time to render services, talking to patients without respect, denial of care, quarantine and verbal/physical abuse has been documented (Brown *et al.,* 2003). These authors showed that 10% of doctors and nurses have admitted having refused to care for an HIV-positive patient or had denied HIV-positive patients admission to a hospital. According to findings of Andrianasolo *et al.,* (2011), 18.5% of HIV patients were denied health care.

The second remarkable manifestation of discrimination is the poor services rendered to PLHIV, estimated to be 8.25%. However this manifestation is almost of same magnitude in both hospitals. Poor services was considered to be: using unnecessary precaution, unwarranted referral to other units or facilities, breach of confidentiality, charging for infection control supplies and addressing in hushed tones. A multivariate analysis to check the factors that predict discrimination: age, gender, level of education and hospital was conducted for all the 4 major manifestations of discrimination. The results showed that there were two times and five times more chance to respectively blame and maltreat patients in Laquintinie than in Nylon (OR: 2.631; 95% CI: 1.319—5.249; p=0.0060) and maltreatment (OR: 5.461; 95% CI: 1.526—19.545; p=0.0091). This can be explained by the workload and the pressure weighing on the nurses in the hospital Laquintinie.

It was found that, 152 participants (76.1%) in the DCH of Laquintinie hospital and 103 participants (51.5 %.) in HCU of Nylon experienced stigmatization. In Kenya it was found that 43% of participants experienced stigma and discrimination (Odindo and Mwanthi*,* 2008). The findings of this study demonstrated that the most cited element (36.8%) of stigmatization was indiscrete manner in which ARV medications were dispensed, that is to say: received in groups of 5-7 at a unit time; given in the presence of other persons not HIV positive patients; waiting room and Day hospital were well known by people as the only site of distribution; involuntary disclosure (calling of names). There was no significant difference in the indiscretion of ARV dispensation in Laquintinie (38.5%) and in Nylon (36%) (p=0.3087). Objective findings showed that there are rarely inward/outward movements into the pharmacy during drug dispensation.

The second element of stigma was the name of the treatment center, which had disrupted 28 patients (7.02%). The reasons being that HIV status is easily deduced because it is reserved for PLHIV only. In this case, all the 28 subjects in Laquintinie were disturbed by the name "Day Hospital" while there none in Nylon. This is justified by the fact that the treatment center in Nylon does not have a particular name like that of Laquintinie. The difference was statistically significant (p <0.001) showing that there is a strong association between the name of the hospital and the discomfort of subjects.

The third stigmatizing element was the conspicuous location of treatment center within the hospital premises being evaluated at 11 % where patients reported to be easily identified as PLHIV. The unit is close to the mortuary with subjects of Laquintinie (12.5%) being more troubled about this than those of Nylon (9.5%) with an insignificant difference between the two hospitals (p=0.3377). The indiscretion of medical files (2.5%,) followed the same trend as they are manipulated by all personnel, involuntary disclosure, signs, exploration of files in the presence of other persons. There was an association between this variable in favor of Laquintinie hospital (p=0.0527).

A total of 59 of interviewees (14.8%) avoided going to the hospital because of previous experience of stigma and discrimination. Some reasons for this motive were: Fear to be identified (7.1%), lack of confidentiality on the part of the personnel (4.7%), fear to be insulted (0.5%), fear to be blamed (0.2%), complain of poor quality services rendered (2.7%), and poor welcome (6.4%). This finding is consistent with that of Campbell *et al.,* (2012), who revealed that perceived and experienced stigma are associated with reduced utilization of prevention services. It was confirmed that experienced and perceived stigma are associated with reduced access to care and treatment by PLHIV (Kinsler *et al.,* 2007).

Also 16 participants (4%) decided to hide their HIV status in case of seeking health care services because of: fear of been identified (2%), fear that their status will be disclosed to others (1%), fear of being verbally abused or harassed (0.7%). The current study agrees with previously published study by Chesney and Smith, (1999), who demonstrated that stigmatization has an effect on health care seeking and strict adherence to medications. The major limitation of this study was the lack of data from the wards and specialized consulting rooms. Additionally, all the elements required to assess stigmatization and discrimination in this study may not have been considered. However, referring to current literature on the subject matter, we took account of the possible variables ever used beforehand. So this study provides important information on determining the extent and impact of stigma and discrimination in a hospital setting.

CONCLUSION

Several difficulties are encountered by patients and particularly those related to stigma and discrimination experienced in a hospital milieu, can constitute obstacles to better therapeutic adherence. The fight against stigma and discrimination should be included in the strategy against the fight against HIV infection by reviewing the management of treatment centers, elaborating relevant public health policies and training sessions.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Adebayo, SB, Bamgbala, AO, Oyediran, MA (2003) Attitudes of Healthcare Providers to Persons Living with HIV/AIDS in Lagos State, Nigeria. Afr J Reprod Health 7 (1): 12- 103.

Andrianasolo, RL, Rakotoarivelo, RA, Randriarimanana, D (2011). Discrimination des personnes infectées par le VIH en milieu médical à Madagascar. Médecine et maladies infectieuses 41: 2–6.

Brown, L, Macintyre, K, Trujillo, L (2003). Interventions to Reduce HIV/AIDS Stigma: What Have We Lear ned? Aids Education and Prevention 15(1): 49-69.

Campbell, C, Skovdal, M, Gibbs, A (2012) Creating Social Spaces to Tackles AIDS- Related Stigma: Reviewing the Role of Church Groups in Sub-Saharan Africa. AIDS Behav15(6): 1204 –1219.

Chan, KY, Stoové, MA, Reidpath, DD (2008) Stigma, social reciprocity and exclusion of people with HIV / AIDS with illicit drugs histories. A study of Thai nurses attitudes. Harm Reduct J 13: 12-28.

Chen, Lai, Xian, Lin, Jayanth Chandran , Hoon, Shim , Wan-Jou, Yang, Huaibin, Cai (2007). The G59S Mutation in p150glued Causes Dysfunction of Dynactin in Mice. Journal of Neuroscience 27(51): 13982-13990.

Chesney, MA, Smith, AW (1999) Critical delays in HIV testing and care: the potential role of stigma. Am Behav Sci 42: 74-114.

Elford, J (2012) HIV-related discrimination reported by people living with HIV in London, UK'. AIDS and Behaviour12: 255- 264.

Feyissa, GT, Abebe, L, Girma, E, Woldie, M (2012) Validation of an HIV-related stigma scale among health care providers in a resource-poor Ethiopian setting. J Multidisciplin Healthcare 5(1):97–113.

Groupe Technique Régional de lutte contre le SIDA pour le Littoral (2012) Rapport deuxième semestre.

Kinsler, JJ, Wong, MD, Sayles, JN (2007) The effect of perceived stigma from a health care provider on access to care among a low-income HIV-positive population. AIDS Patient care Care and STDs21 (8):584-592.

Kremer, H, Ironson, G, Schneiderman, N, Hautzinger, M (2006) To take or not to take: decision-making about antiretroviral treatment in people living with HIV/AIDS. AIDS Patient Care STDS 13:335–349.

Letamo, G (2005) The discriminatory attitudes of health workers against people living with HIV. PLoS Med 2:e261.

Lifson, AR, Demissie, W, Tadesse, A, Ketema, K, May, R, Yakob, B, Metekia, M, Slater, L, Shenie, T (2013) Barriers to retention in care

as perceived by persons living with HIV in rural Ethiopia: focus group results and recommended strategies. J Int Assoc Provid AIDS Care12 (1):32-8.

Mashimo, A, Miura, H, Sakano, S, Hamada, (2001) The role of volunteers of AIDS in the development of community care for people with AIDS in Thailand. Asia Pac J Public Health 13:3-8.

Nguyen, TA, Oosterhoff, P, Pham, YN (2009) Health workers' views on quality of prevention of mother-to-child transmission and postnatal care for HIV-infected women and their children. Human Resources for Health7:39.

Odindo, MA, Mwanthi, MA (2008) Role of governmental and non-governmental organizations in mitigation of stigma and discrimination among HIV/AIDS persons in Kibera, Kenya. East 5(1):1-5.

Oku, AO, Owoaje, ET, Ige ,OK, Oyo-Ita, A(2013) Prevalence and determinants of adherence to HAART amongst PLHIV in a tertiary health facility in south-south Nigeria. BMC Infect Dis13:401.

Oyeyemi, A, Oyeyemi, B, Bello, I (2006) Caring for patients living with AIDS: knowledge, attitude and global level of comfort. Journ of Adv Nur 53:196–204.

Peretti-Watel, P, Spire, B, Obadia, Y, Moatti, JP (2007) Discrimination against HIV-infected people and the spread of HIV: Some evidence from France. PLoS One 2:e411.

Pruss-Ustun, A, Rapiti, E, Hutin, Y (2005) Estimated attributable to contaminated sharps injuries among health care workers global burden of disease. Am Journ of Ind Med 48:482-490.

Tawfik, L, Kinoti, SN (2001) The impact of HIV/AIDS on the health sector in sub-Saharan Africa: the issue of human resources. Washington DC, USA: USAID.

Vanden Driessche, K, Sabue, M, Dufour, W, Behets, F, Van Rie, A (2009) Health workers training to promote HIV services for patients with tuberculosis in the Democratic Republic of Congo. Hum Resour Health13: 23.

Yang, LH, Kleinman, A, Link, BG, Phelan, JC, Lee, S, Good, B (2007) Culture and stigma: adding moral experience to theory stigmatization. Social Science & Medicine. 13:1524-1535.

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ORIGINAL ARTICLE

Anti-inflammatory and anti-oxidant activities of *Secamone afzelii* (Rhoem) Ascleipiadaceae

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Secamone afzelii **is used traditionally in Ghana mainly as a wound healing agent. This study reports the anti-inflammatory and antioxidant properties of *S. afzelii*. The anti-inflammatory activity was determined by the carrageenan-induced paw oedema method in 7 day old chicks and antioxidant property by the 2,2–diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, total antioxidant and total phenol content determinations. In doses of 30, 100 and 300 mg kg^{-1}, the methanol extract of the leaves reduced carrageenan-induced oedema by 24.70%, 33.41% and 44.26% respectively. The leaf extract exhibited free radical scavenging activity with an IC_{50} of 16.73 µg mL^{-1} compared to ascorbic acid which gave an IC_{50} of 2.01 µg mL^{-1}. In the total phenol content determination, the leaf extract was found to contain 56.86 mg g^{-1} calculated as tannic acid equivalent of the dry weight of extract. The extract also demonstrated remarkable antioxidant effect in the total antioxidant capacity determinations and had an 87% correlation with the amount of phenolic content present.**

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Keywords: *Secamone afzelii*, carrageenan, antioxidant, anti-inflammatory

INTRODUCTION

Sub-Saharan Africa is endowed with several medicinal plants with a plethora of historical information for their use in the management of many disease conditions (Lawal *et al*., 2014). Several scientific reports have also justified the biological activities that are traditionally stipulated for these medicinal plants (Agyare *et al*., 2012, Fleischer *et al*., 2013, Kyei *et al*., 2012, Mensah *et al*., 2011, Mensah *et al*., 2001) In recent years, there has been a phenomenal increase in research on medicinal plants used in the in management of inflammatory conditions (Agyare *et al*., 2013, Amponsah *et al*., 2013, Boakye-Gyasi *et al*., 2013, Woode *et al*., 2008) due to severe adverse side effects associated with conventional drugs for the management of inflammation (steroidal and non-steroidal anti-inflammatory drugs). With all efforts therefore geared towards finding suitable herbal remedies for these conditions, studies on *Secamone afzelii* Rhoem (Asclepiadaceae) a creeping woody climber whose aerial parts are appreciated as a medicinal in West Africa (Irvine, 1961) cannot be overlooked. In Ghana, Nigeria and Sierra Leone, the leaves are taken as an infusion, laxative, antispasmodic, analgesic and to treat colic, diarrhoea, urinary tract and sexually transmitted infections. The latex of the leaves is used as a poultice to mature boils and to heal wounds, skin inflammation and breast abscesses. Ground leafy twigs in Shea butter is applied topically to treat nasopharyngeal and respiratory tract infections in children (Kémeuzé, 2010, Burkill, 1985).

Previous studies have shown that *Secamone afzelli* has in vitro antimicrobial activity and the plant extract is able to protect cells against damage by free oxygen radicals (Houghton *et al*., 2005, Mensah *et al*., 2007). The total extract also showed effective free radical scavenging activity in non-enzymatic lipid peroxidation in liposomes which was attributed to α-tocopherol extracted from the leaf (Mensah

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et al., 2004). This study investigates the anti-inflammatory property of the leaf extract of *S. afzelii* using animal models and relates its free radical scavenging and total antioxidant capacity by determining the total phenolic content.

MATERIALS AND METHODS

Plant Collection and Extraction

The leaves of *Secamone afzelii* were collected from the Physique garden of the Faculty of Pharmacy and Pharmaceutical Science, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana in October 2013, authenticated and the voucher specimen, SAS2013 deposited in the herbarium of the Pharmacognosy Department. Two hundred grams (200 g) of the leaves were air dried for 3 days, milled with a mechanical grinder and cold macerated with 500 mL of methanol for 72 hours. The resultant extract was filtered and concentrated to a dark green syrup extract (SA) using a rotary evaporator (R114, Buchi) at temperatures not exceeding 45ºC. The percentage yield was 10.4 % w w^{-1}.

Anti-Inflammatory Assay

Animals

Day old cockerels (*Gallus gallus*) were obtained from Akate Farms, Kumasi, Ghana and were housed in stainless steel cages (34×57×40 cm) at a population density of 10 to 12 chicks per cage. The chicks were fed on chick mash obtained from GAFCO, Tema, Ghana and water *ad libitum*. Temperature was kept at 29°C and overhead incandescent illumination was maintained on a 12 hour light-dark cycle. Chicks were experimented at 7 day-old and were randomly divided into groups of 5 throughout the study.

Experimental design

The various groups of animals comprised the positive control, negative control and treatment groups. Normal saline which was the vehicle for reconstituting the extracts was administered to the negative control, the positive control received 10, 30 and 100 mg kg^{-1} body weight of diclofenac or 0.3, 1 and 3 mg kg^{-1} body weight of dexamethasone and the experimental groups received the *S. afzelii* extract at 30, 100 and 300 mg kg^{-1} body weight. The vehicle and the extracts were administered orally *(p.o)* whiles the standard drugs (diclofenac and dexamethasone) were administered intraperitoneally *(i.p)*.

Carrageenan-induced foot oedema in the chicks

The carrageenan-induced foot oedema model of inflammation in the chick described by Roach and Sufka (2003) with some modification (Boakye-Gyasi *et al.*, 2013, Roach and Sufka, 2003) was used to investigate the anti-inflammatory property of *Secamone afzelii* leaf extract. The foot volume was measured by water displacement plethysmography as described by Fereidoni *et al* (Fereidoni *et al.*, 2000) and carrageenan (10 µl of a 1% suspension in saline) injected sub-plantar into the right footpads of the chicks. Follow up foot volume measurements were then done. Oedema component of inflammation was quantified by measuring the difference in foot volume before carrageenan injection and at an hourly time interval for 5 hours. All experimental protocols were in compliance with the National Institute of Health guidelines for the care and use of laboratory animals and were approved by the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST Ethics Committee.

Antioxidant Assays

2,2–diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity: This assay was performed by the method described by Blois, (1958). Different concentrations of the extract (100 – 6.25 µg ml^{-1}) and ascorbic acid (25 - 0.78 µg ml^{-1}) were used in the assay. The reaction mixture consisted 1ml of each concentration of extract and 3ml of DPPH (20 mg L^{-1}). The mixtures were allowed to stand for 30 minutes and absorbance was measured at 517 nm. Ascorbic acid and blank methanol were taken through the same procedure to serve as positive and negative controls respectively. The percentage of DPPH scavenged was calculated using the following equation:

$$\%\,DPPH\ scavenged = \frac{(A0 - A1)}{(A0)} \times 100$$

Where A_0 = absorbance of negative control, A_1 = absorbance of different concentrations of extract/ ascorbic acid.

Total antioxidant capacity (TAC)

Total antioxidant capacity of the extract was determined as described by Prieto *et al.,* (1999). Different concentrations were prepared for both ascorbic acid (25 - 0.78 µg mL^{-1}) and extract (100 - 6.25 µg mL^{-1}). The reaction mixture consisted of 1ml of plant extract or standard drug with 3 ml of reagent solution (0.6 M H_2SO_4, 28 mM Na_2HPO_4, and 4 mM ammonium molybdate). The mixtures were incubated at 95ºC for 90 minutes and the absorbance determined at 695 nm. The negative control (methanol only) and positive control (ascorbic acid) were treated in the same manner as extracts. The total antioxidant capacity was expressed in terms of ascorbic acid equivalent of the extract (mg g^{-1} of dry mass).

Total phenol content (TPC) determination:

Total phenol content of the extract was determined using the Folin-Ciocalteu's reagent method (Slinkard and Singleton, 1977). Extract (1 ml of 100 - 6.25 µg mL^{-1}) in methanol was separately mixed with 1 ml Folin-Ciocalteu's reagent (1 ml; diluted 1:10 with distilled water) and 1ml of aqueous Na_2CO_3 (2% w v^{-1}, 1 ml) and incubated at room temperature (28ºC) for 2 h. Absorbance was then read at 760 nm using tannic acid as a reference standard. Methanol was processed in the same way and used as blank. The total phenol content was expressed as mg g^{-1} of tannic acid equivalents (TAEs).

Statistical analysis

The raw scores for foot volume increase at each hour (T_1, T_2, T_3, T_4 and T_5) for each animal was normalized as the percentage difference from the initial foot volume at time zero (T_0) and was determined as follows:

$$\%\ Increase\ of\ foot\ volume = \frac{(Foot\ Volume\ at\ T1 - Foot\ Volume\ at\ T0)}{(Foot\ Volume\ T1)} \times 100$$

These values were then averaged for each treatment group and the total foot volume for each treatment group calculated in arbitrary unit as the area under the curve (AUC). Percentage inhibition of oedema for each treatment group was determined as follows:

$$\%\ Inhibition\ of\ oedema = \frac{(AUC\ control - AUC\ treatment)}{(AUC\ control)} \times 100$$

Differences in AUCs were analyzed by one way analysis of variance (ANOVA) followed by Student - Newman Keuls' post test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Secamone afzelii Rhoem (Asclepiadaceae) is used widely in the sub-Saharan region for the treatment of pain, abscess, boils and wounds among others. One major factor underlying most of these diseases is inflammation and associated oxidative stress. In this work, the anti-inflammatory property of the

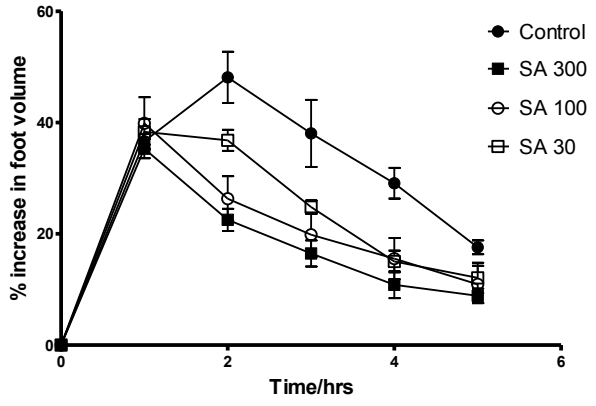

Figure 1a: Time course curve for progression of inflammation for SA (300, 100, 30 mg kg^{-1})

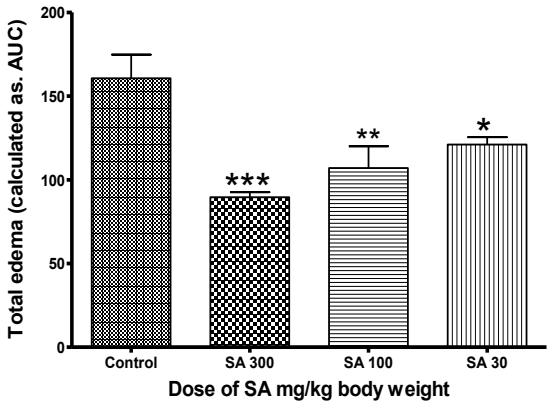

Figure 1b: Total oedema response for SA (300, 100, 30 mg kg^{-1}) calculated as AUCs

leaves of *S. afzelii* was investigated in order to give scientific justification to the reported traditional uses. The total phenol content, total antioxidant capacity as well as and free radical scavenging ability were also investigated. Phytochemical screening of the powdered bark of *S. afzelii,* revealed the presence of bioactive components specifically flavonoids, triterpenes and coumarins which confirms previous works done (Mensah *et al.*, 2004, Mensah *et al.*, 2007, Zabri *et al.*, 2008).

The anti-inflammatory activity was evaluated using the carrageenan-induced paw oedema method in chicks. Carrageenan-induced paw oedema in laboratory animals was first introduced by Winter (Winter *et al.*, 1962) and has been used widely to screen new and potential anti-inflammatory drugs. In this experiment, subcutaneous injection of 1% carrageenan caused a time dependent increase in foot volume which began approximately after one hour of administration and peaked at the third hour. It began to reduce slowly throughout the 5 hour period of the experiment (Figure 1a-3a) indicating the body's owns ability to fight inflammation. However, there was an observable decrease in foot volume which occurred at a faster rate compared to the negative control after ingestion of SA (30-300 mg kg^{-1}) whose effect was dose-dependent. The highest dose of SA gave a 44.26% inhibition of carrageenan induced oedema (Figure 1b) followed by SA 100 mg kg^{-1} (33.41%) and SA 30 mg kg^{-1} (24.70%). The highest effect given by the extract was however lower than that of standard drugs used. The standard drugs used, diclofenac (Figure 2b) and dexamethasone (Figure 3b), showed significant inhibition ($p<0.001$) of oedema at all doses. The highest dose of diclofenac (100 mg kg^{-1}), however gave a better anti-inflammatory effect (71.5±9.3%).

According to Vinegar *et al*, the development of the carrageenan induced inflammation is due to the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin release to the inflamed area (Vinegar *et al.*, 1987). While the anti-inflammatory activity of diclofenac is mediated chiefly through inhibition of the cyclooxygenase pathway (COX 1 and COX 2), particularly prostaglandins and that of dexamethasone is mediated through their suppressive effects on the inflammatory cytokines and on other lipid mediators of inflammation (Enomoto, 2007), the exact mechanism by which *Secamone afzelii* reduces inflammation is not known. It is however possible that the extract may inhibit the release of inflammatory mediators released during carrageenan-induced inflammation.

The antioxidant activity of the methanolic extract of *S. afzelii* leaves was also examined by the DPPH assay, total antioxidant capacity (TAC) and total phenol content (TPC) determination. DPPH is a stable nitrogen-centred free radical with a deep violet colour and absorption maxima at 517 nm. It is

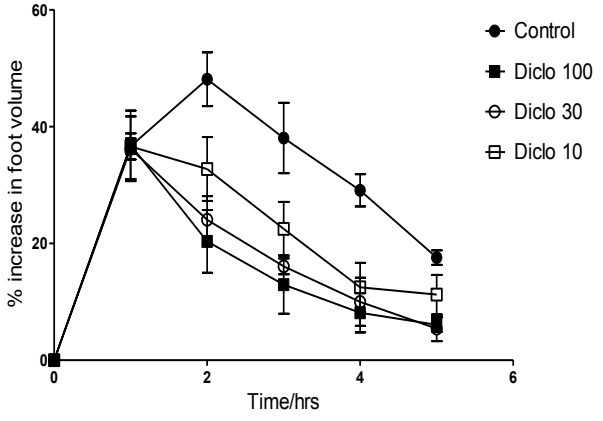

Figure 2a: Time course curve for progression of inflammation for diclofenac (100, 30, 10 mg kg^{-1})

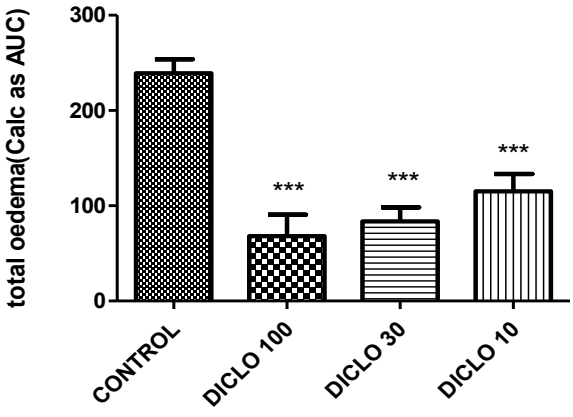

Figure 2b: Total oedema response for diclofenac (100, 30, 10 mg kg^{-1}) calculated as AUCs

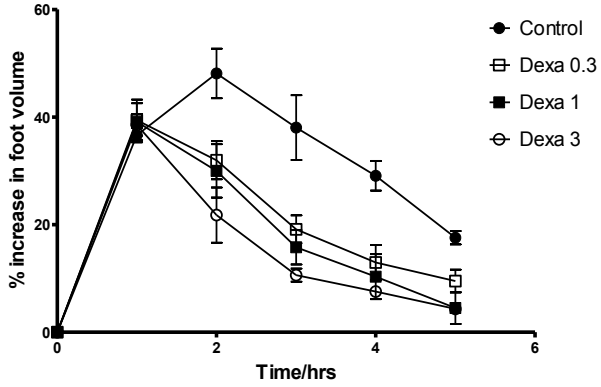

Figure 3a: Time course curve for progression of inflammation for dexamethasone (3, 1, 0.3 mg kg^{-1})

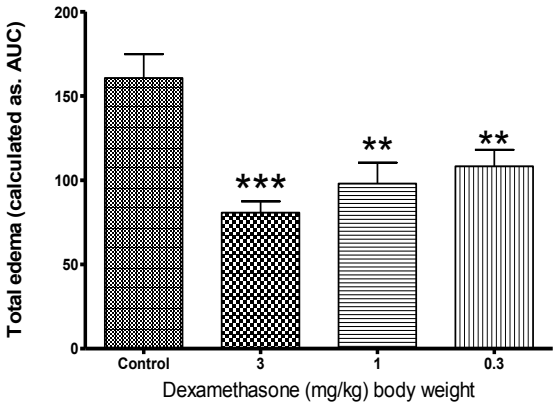

Figure 3b: Total oedema response for dexamethasone (3, 1, 0.3 mg kg^{-1}) calculated as AUCs

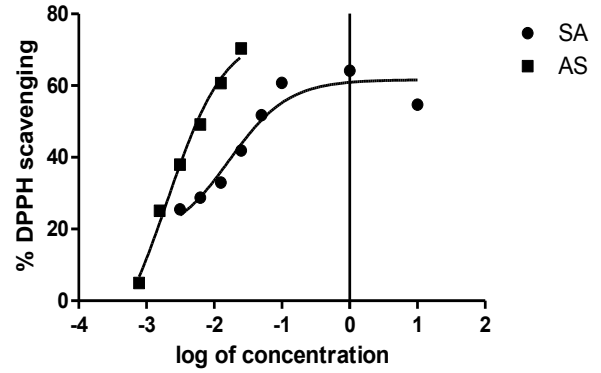

Figure 4: %DPPH absorbance vrs. log concentration of SA extract and ascorbic acid (AS)

Table 1: EC_{50} of SA and ascorbic acid in the DPPH free radical scavenging assay

Drug	EC_{50} (µg mL^{-1})
Secamone afzelii	16.73
Ascorbic acid	2.018

decolorized when it accepts an electron from the antioxidant compound to form DPPH-H. The amount of residual DPPH is quantitatively measured from changes in UV absorbance at 517 nm (Blois, 1958). The methanolic extract (SA) caused a dose dependent decrease in DPPH absorbance, with an EC_{50} of 16.73 µg mL^{-1}. This effect was lower than that of the standard drug, ascorbic acid which had an EC_{50} of 2.01 µg mL^{-1} (Figure 4; Table 1).

The phosphomolybdate method was used to evaluate the total antioxidant capacity (TAC) of the extract. The TAC was expressed as the number of equivalents of ascorbic acid in mg g^{-1} of the dry mass of extract. It is based on the reduction of Mo (VI) to Mo (V) by the antioxidant agent (i.e. *Secamone afzelii* or ascorbic acid) and the formation of a green phosphate complex (Athukorala *et al.*, 2006). An increased amount of complex compound formed causes an increase in absorbance and indicates better antioxidant capacity. The results of the experiment indicated that *Secamone afzelii* has antioxidant activity which increases with increasing concentration. The highest TAC recorded for *S. afzelii* leaves was 112.86 mg g^{-1} (ascorbic acid equivalent). The total anti-oxidant capacity increase with increasing concentration of the extract (Figure 5).

The presence of phenolic compounds and flavonoids in plants has been associated with their antioxidant action in biological systems (Chanda and Dave, 2009). Due to their structural chemistry, plant phenols act as hydrogen or electron donors to stabilize the unpaired radical (Rice-Evans *et al.*, 1997). During the inflammatory process, the excessive production of reactive oxygen metabolites by

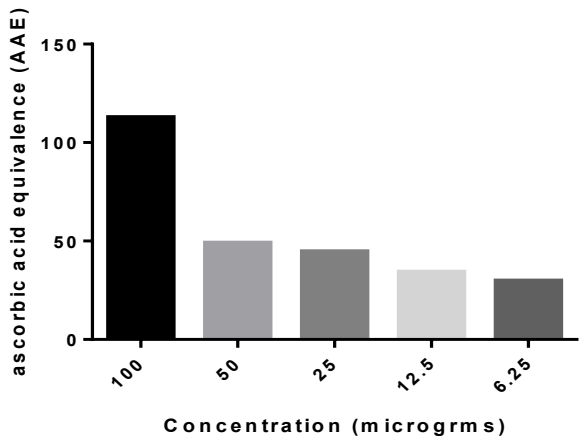

Figure 5: Total antioxidant capacity of *S. afzelii* measured as ascorbic acid equivalent in mg/g at different concentrations of extract.

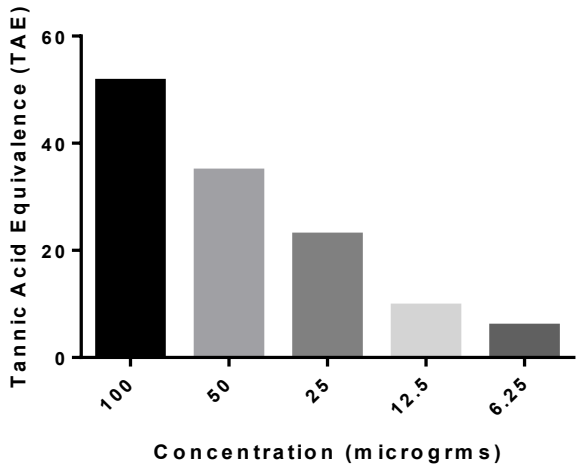

Figure 6: Total phenolic content of *S. afzelii* measured as tannic acid equivalent in mg g^{-1} at different concentrations of extract.

phagocytic leucocytes causes tissue injury which in turn augments the state of inflammation and lead to chronic inflammatory states. Antioxidants, which scavenge these reactive oxygen metabolites, have been found to complement the anti-inflammatory process, enhance tissue repair and promote wound healing (Wu *et al.*, 2006, Vinegar *et al.*, 1987). From the results, *Secamone afzelii* contains phenols. The TPC was 56.86 mg g^{-1} of dry mass of extract ex-

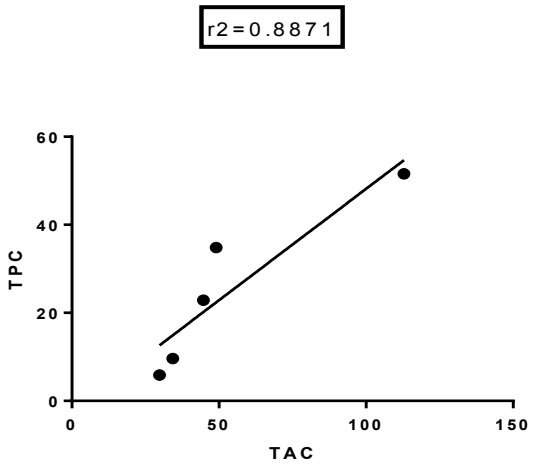

Figure 7: Correlation between TPC and TAC

pressed as the number of equivalent tannic acid. As concentration of the extract increase, there was an observable increase in absorbance corresponding to an increase in phenolic content (Figure 6). A linearity graph of TPC against TAC was also obtained to show the relationship between the phenol content of the extract and its antioxidant capacity (Figure 7). From the linearity graph, 88.7% of its antioxidant activity is due to the presence of phenols and the remaining 12% from other components of the plant. The antioxidant activity of the extracts may therefore partly contribute to its anti-inflammatory activity because inflammatory tissue injuries are mediated by reactive oxygen metabolites from phagocytic leucocytes that invade the tissue (Zaikov, 2000). The antioxidant activity of the plant may support its traditional use in Ghana as a wound healing agent.

CONCLUSION

The anti-inflammatory activity observed for *S. afzelii* gives some scientific justification for its use in the treatment of inflammatory conditions and wounds. The presence of phenolic matter and antioxidant activity is further confirmed and may be involved in the wound healing process of the plant.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Agyare, C., Dwobeng, A. S., Agyepong, N., Boakye, Y. D., Mensah, K. B., Ayande, P. G. & Adarkwa-Yiadom, M. 2013. Antimicrobial, Antioxidant, and Wound Healing Properties of *Kigelia africana* (Lam.) Beneth. and *Strophanthus hispidus* DC. *Advances in pharmacological sciences*, 1-10.

Agyare, C., Koffuor, G. A., Boamah, V. E., Adu, F., Mensah, K. B. & Adu-Amoah, L. 2012. Antimicrobial and anti-inflammatory activities of *Pterygota macrocarpa* and *Cola gigantea* (Sterculiaceae). *Evidence-Based Complementary and Alternative Medicine*, 1-10.

Amponsah, I. K., Fleischer, T. C., Dickson, R. A., Annan, K. & Thoss, V. 2013. Evaluation of anti-inflammatory and antioxidant activity of Furanocoumarins and Sterolin from the stem bark of *Ficus exasperata* Vahl (Moraceae). *Journal of Scientific and Innovative Research,* 2**,** 880-887.

Athukorala, Y., Kim, K. N. & Jeon, Y. J. 2006. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food and Chemical Toxicology,* 44**,** 1065-1074.

Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical.

Boakye-Gyasi, E., Abotsi, W. K. & Woode, E. 2013. Anti-inflammatory and analgesic effects of ethanolic leaf extract of *Newbouldia laevis* (P. Beauv.) Seemann Ex Bureau (Bignoniaceae). *Der Pharmacia Sinica,* 4.

Burkill, H. 1985. *The useful plants of west tropical Africa. Edition 2. Vol. 1: families AD*.

Chanda, S. & Dave, R. 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research,* 3**,** 981-996.

Enomoto, R., Suzuki, C., Koshiba, C., Nishino, T., Nakayama, M., Hirano, H., Yokoi, T. & Lee, E. 2007. Wogonin prevents immunosuppressive action but not anti-inflammatory effect induced by glucocorticoid. *Annals of the New York Academy of Sciences,* 1095**,** 412-417.

Fereidoni, M., Ahmadiani, A. & Samnanian, S. 2000. An accurate and simple method for measurement of paw oedema. *Journal of Pharmacology and Toxicological Methods* 43**,** 11 -14.

Fleischer, T. C., Annan, K., Dickson, R. A., Mensah, A. Y. & Sarpong, F. M. 2013. Anti-inflammatory, antioxidant and antimicrobial activity of the stem bark extract and fractions of *Ficus exasperata* Vahl. (Moraceae). *Journal of Pharmacognosy and Phytochemistry,* 2**,** 38-44.

Houghton, P. J., Hylands, P. J., Mensah, A. Y., Hensel, A. & Deters, A. M. 2005. In vitro tests and ethnopharmacological investigations: wound healing as an example. *Journal of Ethnopharmacology,* 100**,** 100-107.

Irvine, F. R. 1961. Woody plants of Ghana. *Woody plants of Ghana*.

Kémeuzé, V. A. 2010. *Secamone afzelii* (Schult.) K.Schum; In: Schmelzer, G.H. & Gurib-Fakim, A.(Editors). *Medicinal plants/Plantes médicinales 2*. Wageningen, Netherlands: PROTA, .

Kyei, S., Koffuor, G. A. & Boampong, J. N. 2012. Antiarthritic effect of aqueous and ethanolic leaf extracts of *Pistia stratiotes* in adjuvant-induced arthritis in Sprague-Dawley rats. *Journal of Experimental Pharmacology,* 4**,** 41-51.

Lawal, I., Grierson, D. & Afolayan, A. 2014. Phytotherapeutic Information on Plants Used for the Treatment of Tuberculosis in Eastern Cape Province, South Africa. *Evidence-Based Complementary and Alternative Medicine*.

Mensah, A. Y., Donkor, P. O. & Fleischer, T. C. 2011. Anti-Inflammatory And Antioxidant Activities Of The Leaves Of *Wissadula Amplissima* Var Rostrata. *African Journal of Traditional, Complementary and Alternative Medicines,* 8.

Mensah, A. Y., Houghton, P. J., Agyare, C., Komlaga, G., Mensah, M. L. K., Fleisher, T. C. & Sarpong, K. 2007. Investigation of Activities Related to Wound Healing of *Secamone Afzelii*. *Journal of Science and Technology (Ghana),* 26**,** 83-89.

Mensah, A. Y., Houghton, P. J., Akyirem, G. N. A., Fleischer, T. C., Mensah, M. L. K., Sarpong, K. & Adosraku, R. 2004. Evaluation of the antioxidant and free radical scavenging properties of *Secamone afzelii* Rhoem. *Phytotherapy Research,* 18**,** 1031-1032.

Mensah, A. Y., Sampson, J., Houghton, P. J., Hylands, P. J., Westbrook, J., Dunn, M., Hughes, M. A. & Cherry, G. W. 2001. Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. *Journal of Ethnopharmacology,* 77**,** 219-226.

Prieto, P., Pineda, M. & Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry,* 269**,** 337-341.

Rice-Evans, C., Miller, N. & Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in plant science,* 2**,** 152-159.

Roach, J. T. & Sufka, K. J. 2003. Characterization of the chick carrageenan response. *Brain research,* 994**,** 216-225.

Slinkard, K. & Singleton, V. L. 1977. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture,* 28**,** 49-55.

Vinegar, R., Truax, J. F., Selph, J. L., Johnston, P. R., Venable, A. L. L. & Mckenzie, K. K. 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Federation proceedings.*

Winter, C. A., Risley, E. A. & Nuss, G. W. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. *Proc Soc Exp Biol Med,* 111**,** 544-7.

Woode, E., Ansah, C., Ainooson, G. K., Abotsi, W. M., Mensah, A. Y. & Duweijua, M. 2008. Anti-inflammatory and antioxidant properties of the root extract of *Carissa edulis* (Forsk.) Vahl (Apocynaceae). *Journal of Science and Technology (Ghana),* 27**,** 5-15.

Wu, S. J., Tsai, J. Y., Chang, S. P., Lin, D. L., Wang, S. S., Huang, S. N. & Ng, L. T. 2006. Supercritical carbon dioxide extract exhibits enhanced antioxidant and anti-inflammatory activities of *Physalis peruviana. Journal of ethnopharmacology,* 108**,** 407-413.

Zabri, H., Kodjo, C., Benie, A., Bekro, J. M. & Bekro, Y. A. 2008. Phytochemical screening and determination of flavonoids in *Secamone afzelii* (Asclepiadaceae) extracts. *African Journal of Pure and Applied Chemistry,* 2**,** 080-082.

Zaikov, G. E. 2000. *Biotic type antioxidants: The prospective search area for novel chemical drugs*, VSP.

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ORIGINAL ARTICLE

Biochemical and haematological changes following an acute toxicity study of a hydro-ethanolic whole plant extract of *Synedrella nodiflora* (L) Gaertn in male Sprague-Dawley rats

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***Synedrella nodiflora* (L) Gaertn (family Asteraceae), a common weed in Ghana, is traditionally used for the management of epilepsy, hiccup and threatened abortion. To further promote the ethnopharmacological uses of the plant, an acute toxicity of a hydro-ethanolic whole plant extract was assessed in male Sprague-Dawley rats. The lethal dose (LD_{50}) and effects of a single oral administration of the extract (1600, 3200 and 6400 mg kg^{-1}) on haematological and serum biochemical parameters were measured. The extract produced no mortality in the rats treated during a 48-hour examination and after a subsequent 12-day assessment. Thus the LD_{50} was indicated as being greater than 6400 mg kg^{-1}. The extract also did not significantly affect any of the haematological and serum biochemical indices. This result suggests that acute oral administration of the hydro-ethanolic extract of *Synedrella nodiflora* is virtually non-toxic in male Sprague-Dawley rats under normal laboratory conditions.**

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Keywords: *Synedrella nodiflora,* acute toxicity, Sprague-Dawley rats, haematology, biochemical analysis

INTRODUCTION

Synedrella nodiflora (L.) Gaertn (family Asteraceae) is a common weed of waste places and found along the banks of rivers, streams and also along roadsides (Mshana *et al.,* 2000). In Ghana, the whole plant is boiled and the aqueous extract drunk for the treatment of epilepsy whiles the leaves are used for threatened abortion, hiccup, laxative and feed for livestock (Dalziel, 1931; Mshana *et al.,* 2000). The plant is also used by subsistence farmers of Ghana as post-harvest protectants (Cobbinah *et al.,* 1999). Traditional uses of the plant in other African and some Asian countries have been reported. In Nigeria, it is known that some indigenous tribes use the whole plant for the treatment of cardiac distresses and to stop wound bleeding (Idu and Onyibe, 2007). The foliage is readily eaten by livestock in Cameroon (Irvine, 1961). In Indonesia the young foliage is eaten as a vegetable and the leaf sap together with other materials, is applied for stomachache and the plant is used in embrocation for rheumatism (Burkill, 1985). In Malaysia, a poultice of the leaves are used for managing sore legs and for the treatment of headache and the sap is instilled into the ear for earache (Burkill, 1985).

The hydro-ethanolic extract of the whole plant has been found to possess anticonvulsant (Amoateng *et al.,* 2012), sedative (Woode *et al.,* 2011), *in vitro* antioxidant and free radical scavenging properties (Amoateng *et al.,* 2011) and anti-nociceptive properties (Woode *et al.,* 2009). To explore the plant for further pre-clinical anti-epileptic drug discovery and development, it is important to investigate the toxicity of the plant. The leaf extract of *S. nodiflora*, among other plants investigated, has been reported

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to control storage pests but had no toxic effect in vertebrates (Belmain *et al.,* 2001). The insecticidal effects of various solvent extracts of the aerial parts of *S. nodiflora* on the fourth instar larvae of *S. litura* has also been reported (Martin and Gopalakrishnan, 2005). To clarify the extent to which the plant is toxic in rodents, the present study reports an acute toxicity of the hydro-ethanolic extract of the whole plant of *Synedrella nodiflora* in male Sprague-Dawley rats.

MATERIALS AND METHODS

Plant collection and extraction

Samples of the plant were collected from the Botanical Gardens, University of Ghana, Accra in August 2012 and were identified and authenticated at Ghana Herbarium, Department of Botany, University of Ghana, Legon, Accra where a voucher specimen (# PA01/UGSOP/GH12) was kept. The hydro-ethanolic extract was prepared as previously described by Woode *et al.,* (2011). Briefly, the samples of the collected plant were air-dried for seven days and powdered. Suitable amounts of the powder were cold-macerated with 70% v/v of ethanol in water. The hydro-ethanolic extract was then evaporated to a syrupy mass under reduced pressure, air-dried, kept in a dessicator and the percent yield calculated. The resultant product was subsequently referred to as the extract or SNE.

Phytochemical screening of SNE

The hydro-ethanolic extract was tested qualitatively for the presence of flavonoids, tannins, saponins, sterols, alkaloids, cardiac glycosides, coumarins, triterpenoids, anthraquinones and phenolic compounds based on test methods as previously described by Evans (2001).

Animals

Male Sprague-Dawley (SD) rats (150-200 g), 6-8 weeks old were obtained from the Animal Experimentation, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Accra. The animals were housed in groups of five in stainless steel cages (34 cm x 47 cm x 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (AGRIMAT, Kumasi), given water *ad libitum* and maintained under laboratory conditions (temperature 22 ± 2°C, relative humidity 60-70% and 12 hour light-dark cycle) for seven (7) days prior to the acute toxicity study. All animal procedures and techniques used in these studies were in accordance with the Noguchi Institute Animal care and use committee (NIACUC) guidelines as well as the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83-23, revised 1985).

Animal Groupings and Acute extract administration

The acclimatized SD rats were randomly grouped into four (five rats/group) namely; vehicle (distilled water 1.667ml kg^{-1}), SNE 1600 mg kg^{-1}, SNE 3200 mg kg^{-1} and SNE 6400 mg kg^{-1}. The vehicle and SNE were administered orally (by gavage) to mimic the traditional folkloric route of administration.

48 hour Clinical Observations and LD_{50} determination

After administration of the extract/distilled water, the animals in each group were observed every seven hours for clinical signs of toxidromes such as changes in movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool and mortality within forty-eight hours. Mortality after twenty-four and forty-eight hour post treatment were recorded and the LD_{50} (the lethal dose) was determined.

A 12-day Clinical Observation and acute toxicity study

The animals were then monitored and observed daily during the next 12 days for any clinically observed toxidromes and mortality. On the 14th day of the study period the rats were euthanized and blood samples were collected from each animal via cardiac puncture into BD microtainer brand tube with EDTA (1 ml) and BD vacutainer SST – II Advance (5 ml) for haematological and biochemical analysis, respectively. An automated haematology analyzer (KX-2IN, Sysmex Corporation, Japan) was used for the haematological analysis and Selectra Junior version 04 autoanalyzer (Vital Scientific Bv, Nether-

lands) for the biochemical assays (renal function (urea, creatinine, potassium and sodium), lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol) and liver function test (total protein, albumin, globulin, direct, indirect and total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzyme assays).

The animals were immediately autopsied and all visible organs and tissues were macroscopically examined, harvested and stored in formalin. A gross necropsy was performed and post-mortem examinations conducted.

Data Analysis
GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. $P \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Phytochemical screening of SNE
SNE as screened for the presence of various phytochemical constituents produced evidence for the presence of the following: flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarins, triterpenes, sterols, anthraquinones and phenols.

Clinical Observations
The single oral administration of SNE (1600, 3200 and 6400 mg kg^{-1}) did not produce observable abnormality in the movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool of rats in comparison to the vehicle-treated group within the first 48-hours and daily for the rest of the 14-day of the study period .

LD_{50}
After monitoring the animals for 48 hours and a further 12 days, SNE (1600-6400 mg kg^{-1}, *p.o*) yielded no deaths. Hence it can be said that the LD_{50} of the extract when orally administered is greater than 6400 mg kg^{-1}.

Post-mortem Observations
A post-mortem examination of the SNE-and vehicle-treated rats revealed no visible abnormal effect in all major organs observed.

Haematological and Biochemical Analysis
There was no significant difference (P=0.26-0.56) between the vehicle-treated group and the extract (1600, 3200 and 6400 mg kg^{-1}) regarding all the haematological indices measured (Table 1).

The serum biochemical markers were grouped as: renal function (urea, creatinine, potassium and sodium), lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol) and liver function test (total protein, albumin, globulin, direct, indirect and total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzyme assays) and presented in Table 2. Regarding the renal function of the rat subjects, there was no significant difference (P=0.10-0.97) between the vehicle-treated and SNE (1600, 3200, 6400 mg kg^{-1})-treated rats. Similarly there was no significant (P=0.25-0.61) changes in the lipid profile of SNE-treated rats in comparison to vehicle-treated rats. With respect to the liver function assessment of the rats used in the study, there was no significant difference (P=0.28-0.83) between the vehicle-treated and the SNE-treated rats for all parameters measured.

DISCUSSION

The present study presents an acute toxicity of a hydro-ethanolic extract of *Synedrella nodiflora* in male SD rats. The single oral administration of increasing doses of the extract (1600, 3200 and 6400 mg kg^{-1}) was generally found to be less toxic and produced no mortality in the rats during the entire study period. The absence of any statistically significant changes in haematological, biochemical and gross organ assessments of rats treated with the extract provides support for the safety of the extract.

Table 1: Haematological analysis of a single administration of SNE (1600, 3200 and 6400 mg kg^{-1}) after a 14-day observation period in SD male rats

Parameter	Vehicle	SNE 1600	SNE 3200	SNE 6400	P value
WBC (10^3 µL^{-1})	11.98 ± 1.41	10.14 ± 1.22	11.00 ± 0.57	10.28 ± 1.52	0.71
RBC (10^6 µL^{-1})	6.99 ± 0.31	7.40 ± 0.15	7.28 ± 0.22	7.50 ± 0.18	0.44
HGB (g dL^{-1})	13.24 ± 0.66	14.00 ± 0.29	13.94 ± 0.50	14.00 ± 0.10	0.56
HCT (%)	42.26 ± 2.02	44.98 ± 1.10	44.08 ± 1.77	44.80 ± 0.60	0.56
MCV (fl)	60.40 ± 0.62	60.78 ± 0.49	60.52 ± 1.2	59.86± 1.24	0.92
MCH (pg)	18.94 ± 0.20	18.94 ± 0.08	19.14 ± 0.22	18.72± 0.39	0.71
MCHC (gdL^{-1})	31.34 ± 0.13	31.12 ± 0.14	31.64 ± 0.28	31.26± 0.35	0.51
PLT (10^3 µL^{-1})	813.60 ± 171.70	687.20 ± 224.80	816.40 ± 181.60	790.60 ± 103.80	0.95
LYM (%)	88.14 ± 1.37	90.18 ± 0.76	91.42 ± 1.141	88.62± 1.73	0.30
NEUT (%)	6.380 ± 0.47	6.34 ± 0.97	5.22 ±0.83	7.28± 0.95	0.41
LYM (10^3 µL^{-1})	11.44 ± 1.32	9.12 ± 1.09	9.00 ±1.14	7.84± 1.73	0.32
NEUT (10^3 µL^{-1})	0.82 ± 0.10	0.64 ± 0.13	0.56± 0.11	0.62± 0.12	0.45
RDW_SD (fl)	28.62 ± 0.39	28.66 ± 0.54	28.72 ± 0.70	28.30 ± 0.51	0.95
RDW_CV (%)	10.04 ± 0.09	10.04 ± 0.44	10.24 ± 0.32	9.98 ±0.28	0.94
PDW (fl)	7.68 ± 0.21	7.60 ± 0.27	8.18 ±0.32	7.90 ±0.33	0.52
MPV (fl)	6.64 ± 0.11	6.60 ± 0.19	6.86 ±0.16	6.76 ±0.18	0.67
P_LCR (%)	5.38 ± 0.67	4.90 ± 0.91	6.06 ±0.98	5.44 ±0.83	0.83

Table 2: Biochemical analysis of a single administration of SNE (1600, 3200 and 6400 mg kg^{-1}) after a 14-day observation period in SD male rats

Parameters	Vehicle	SNE 1600	SNE 3200	SNE 6400	P value
Renal function test (mmol L^{-1})					
Urea	8.74 ± 0.22	8.08 ± 0.34	9.00 ± 0.23	8.52 ± 0.20	0.10
Creatinine	57.38 ± 5.07	58.48±3.99	51.48±4.23	59.24 ± 2.69	0.54
Potassium	3.34 ± 0.11	3.65 ± 0.12	3.20 ± 0.02	3.56 ± 0.16	0.15
Sodium	141.4 ± 0.24	141.4 ± 1.81	141.40 ± 0.24	140.8 ± 1.02	0.97
Lipid Profile (mmol L^{-1})					
Total Cholesterol	2.11 ± 0.14	2.02 ± 0.08	2.22 ± 0.13	2.20 ± 0.11	0.61
Triglycerides	0.65 ± 0.10	0.46 ± 0.09	0.62 ± 0.09	0.71 ± 0.16	0.48
HDL	1.03 ± 0.04	1.02 ± 0.04	1.01 ± 0.04	1.02 ± 0.04	0.99
LDL	0.82 ± 0.07	0.85 ± 0.02	1.17 ± 0.24	0.86 ± 0.11	0.25
VLDL	0.29 ± 0.05	0.21 ± 0.04	0.28 ± 0.04	0.32 ± 0.07	0.49
Liver function test					
Total Protein (g L^{-1})	63.50 ± 1.50	63.08 ± 2.14	63.85 ± 0.55	65.44 ± 1.18	0.71
Albumin (gL^{-1})	34.68 ± 0.58	33.88 ± 0.99	33.86 ± 0.62	34.96 ± 0.63	0.62
Globulin (gL^{-1})	28.80 ± 1.05	29.16 ± 1.22	16.48 ± 13.74	30.50 ± 0.95	0.47
D. Bilirubin (µmolL^{-1})	1.66 ± 0.10	2.62 ± 0.73	1.66 ± 0.09	1.84 ± 0.20	0.28
Ind. Bilirubin (µmolL^{-1})	0.52 ± 0.15	1.20 ± 0.62	2.02 ± 1.73	0.66 ± 0.18	0.66
T. Bilirubin (µmolL^{-1})	2.18 ± 0.22	3.14 ± 0.71	3.93 ± 0.16	2.50 ± 0.38	0.05
ALT (UL^{-1})	33.55 ± 1.65	33.20 ± 1.23	30.30 ± 0.40	34.43 ± 2.87	0.60
AST (IUL^{-1})	18.08± 2.95	11.18± 2.76	22.58± 2.53	12.43± 1.93	0.03
ALP (UL^{-1})	51.34 ± 4.17	46.53 ± 4.16	38.90 ± 2.90	51.02 ± 6.99	0.26

The determination of the lethal dose (LD_{50}) of an oral administration of the hydro-ethanolic extract of *Synedrella nodiflora* (SNE) in this study was paramount. The LD_{50} as determined to be greater than 6400 mg kg^{-1} indicates that SNE is relatively safe. The highest therapeutic dose of the hydro-ethanolic extract demonstrating anticonvulsant and related neuropharmacological properties is lower (i.e. 1000 mg kg^{-1}) (Woode *et al.,* 2009, Amoateng *et al.,* 2011, Amoateng *et al.,* 2012), than the doses used in this study (which are 1600, 3200 and 6400 mg kg^{-1}). Thus this present study only demonstrates the effects of acute administration of high doses of the extract beyond 1000 mg kg^{-1}. Also the extract produced no untoward change in the metabolic and physical behaviour of rats treated in comparison to the vehicle-treated rats. This confirms previous reports that the plant extract is relatively safe in rodents (Belmain *et al.,* 2001). More so, since the leaves of the plant is eaten as food with no known documented or reported adverse effects, the plant extract can also be said as being less toxic in humans as well.

Haemotoxicants such as paracetamol and some phytochemicals are known to reduce red blood cell counts and also affect the haemoglobin concentration and subsequently produce anaemia in experimental animals (Mullick *et al.*, 1973; Patrick-Iwuanyanwu *et al.*, 2007). The haematological parameters as measured in the various doses of SNE in the SD rats failed to produce any significant difference in comparison to vehicle-treated rats, suggesting that the extract possess no deleterious effects on blood and blood-forming cells. Moreso, it may be inferred that the extract may have no haematinic, immunosuppressive or stimulatory properties in rats.

On the renal function of the SD rats, the extract did not produce any significant effect on the biochemical parameters (urea, creatinine, sodium and potassium). These suggest the extract may be devoid of any effect whether therapeutic or adverse confirm again that the extract is less toxic in rodent (Belmain *et al.,* 2001). Furthermore, the extract did not significantly affect the lipid profile (total cholesterol, HDL, LDL and VLDL cholesterols) of the SD rats. This suggests that the extract may not have any potential therapeutic or adverse effect on lipid metabolism.

Liver function was determined by the measurement of well-known liver enzyme markers (ALT, ALP and AST) as well as direct, indirect and total bilirubin, elevations of which may suggest a liver and/ bile duct damage as well as enhanced haemoglobin breakdown (Corns, 2003; Arneson and Brickell, 2007; Odetola, 2012). These measurements were done primarily to detect possible hepatic dysfunction, tissue damage or changes in biliary excretion induced by a single oral administration of high doses of SNE. Since there were no significant changes in liver enzymes following the administration of SNE, it can be inferred that the extract did not induce any hepatic damage (Chalasani *et al.,* 2012). Similarly, since there was no significant change in direct, indirect and total bilirubin after treatment with SNE, it also indicates that the extract did not alter hepatic metabolism or biliary secretions. An overall assessment of the effect of the extract on the liver coupled with no visible macroscopic abnormalities generally suggests that the extract may have no liver toxicity much especially when used below 1600 mg kg^{-1} for therapeutic purposes. However, a sub-acute, sub-chronic or chronic assessment of the therapeutic doses of the extract should be performed to conclude this assertion.

The hydro-ethanolic extract was found to possess flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarins, triterpenes, sterols, anthraquinones and phenols confirming previous reports (Amoateng *et al.,* 2012). Since no untoward effects of the extract in rats were found, it could be said that these phytoconstituents as found in the extract may be relatively nontoxic in rodents. However this assertion should be further clarified by other toxicological assessments of the whole extract as well as isolation and toxicological characterization of these phytoconstituents.

CONCLUSION

The hydro-ethanolic extract of *Synedrella nodiflora* (L) Gaertn has an oral LD_{50} being greater than 6400 mg kg^{-1} and no significant effect on the haematological and biochemical parameters in male SD rats.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Amoateng, P., Assumeng Koffuor, G., Sarpong, K., Oteng Agyapong, K.(2011). Free Radical Scavenging and Anti-lipid Peroxidative Effects of a Hydro-ethanolic Extract of the Whole Plant of Synedrella nodiflora (L.) Gaertn (Asteraceae). *Free Rad Antiox*,1:70-8.

Amoateng, P., Woode, E., Kombian, S.B.(2012). Anticonvulsant and related neuropharmacological effects of the whole plant extract of Synedrella nodiflora (L.) Gaertn (Asteraceae). *J Pharm Bioall Sci*,4:140-8.

Arneson, W., Brickell, J.(2007). Assessment of liver function, Clinical Chemistry: A Laboratory Perspective. 1st ed. Philadelphia: F.A. Davis Davis Company.

Belmain, S.R., Neal, G.E., Ray, D.E., Golob, P. (2001) Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food Chem Toxicol*, 39:287-91.

Burkill, H.M.(1985). The Useful Plants of West Tropical Africa. 2 ed: Royal Botanical Gardens Kew.

Chalasani, N., Younossi, Z., Lavine, J.E.(2012). The diagnosis and management of non-alcoholic fatty liver disease practice guidelines by the American Gastroenterological Association, American Association for the study of Liver Diseases, and American College of Gastroenterology. *Gastroenterol*, 142:1592.

Cobbinah, J.R., Moss, C., Golob, P., Belmain, S.R. (1999). Conducting ethnobotanical surveys; an example from Ghana on plants used for the protection of stored cereals and pulses. NRI Bulletin.

Corns, C.M.(2003). Herbal remedies and clinical biochemistry. *Ann Clin Biochem*, 40:489-507.

Dalziel, J.M.(1931). The hairs lining the loculi of fruits of species of Parinarium. *Proc Linn Soc London*:99.

Evans, W.C. (2001). Trease and Evan's Pharmacognosy. 15 ed. London: Bailliere Tindall.

Idu, M., Onyibe, H.I.(2007). Medicinal Plants of Edo State,Nigeria. *Res J Med Plant*,1:32-41.

Irvine, F.R. (1961). Woody plants of Ghana. London: Oxford University Press.

Martin, R.J., Gopalakrishnan, S.(2005). Insecticidal Activity of Aerial Parts of Synedrella Nodiflora Gaertn (Compositae) on Spodoptera Litura (Fab.). *J Central Eur Agric*, 6:223-8.

Mshana, N.R., Abbiw, D.K., Addae-Mensah, I., Adjanohoun, E., Ahyi, M.R.A., Enow-Orock, E.G., et al.(2000). Traditional Medicine and Pharmacopoeia. Contribution to the revision of ethnobotanical and floristic studies in Ghana. Scientific, Technical and Research Commission of the Organization of African Unity.

Mullick, F.G., Delage, C., Irey, N.S. (1973). Sickle cell crisis associated with drugs. *Arch Environmental Health*; 26:221-222.

Odetola, M.O., Ewuola, E.O., Adu, O.A.(2012). Haematology, serum biochemistry and organ histopathology of rabbits fed graded levels of whole Kenaf (*Hibiscus canabinus*) seed meal. *Int J Agric Res*, 7:86-92.

Patrick-Iwuanyanwu, K.C., Wegwu, M.O., Ayalogu, E.O. (2007). Prevention of CCl4-induced liver damage by ginger, garlic and vitamin E. *Pakistan J Biol Sci*, 10:617-621.

Woode, E., Amoateng, P., Abotsi, W.M.K. (2011). Ethopharmacological analysis of the effects of the whole plant extract of Synedrella nodiflora (L.) Gaertn (Asteraceae) in murine models. *Der Pharmacia Sinica*, 2:54-67.

Woode, E., Amoateng, P., Ansah, C., Duwiejua, M. (2009). Anti-Nociceptive Effects of an Ethanolic Extract of the Whole Plant of Synedrella nodiflora (L.) Gaertn in Mice: Involvement of Adenosinergic Mechanisms. *J Pharm Toxicol*, 4:17-29.

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