Journal of Medical and Biomedical Sciences

VOLUME 06, ISSUE 2, APRIL 2017

ORIGINAL ARTICLES

Journal of Medical and Biomedical Sciences (2018) 7(2): 1-9

ORIGINAL ARTICLE

Impact of physical activity levels and diet on central obesity among civil servants in Tamale metropolis

V. Mogre[1], P. P. Mwinlenaa[2], J. Oladele[2] and A. Amalba[1]

[1]Department of Human Biology, [2]Department of Community Nutrition, School of Medicine and Health Sciences, University for Development Studies, Tamale, Ghana

This study sought to assess the prevalence of central obesity using Waist to Hip ratio as well as establishing the relationship between physical activity, diet and central obesity among civil servants in the Tamale metropolis. This cross-sectional study was conducted between January and July, 2011. One hundred and eighty six (186) subjects were involved with 121 being males and 65 being females. The study participants were recruited from an adult population between the ages of 20 -59 years. Dietary pattern was assessed using food frequency questionnaires whilst physical activity was determined using the WHO Global physical activity levels questionnaire. The prevalence of central obesity was found to be 31.2%. The prevalence of central obesity was significantly higher (p=0.031) among females (41.5%) compared to men (25.6%) and also increased with age (p<0.0001). The prevalence of obesity significantly decreased (p = 0.018) from 70.7% via 25.6% to 3.4% as the level of physical activity increased from low through moderate to high. There were no significant associations between dietary pattern and central obesity from this study. Central obesity from this study is high and more common in females. The level of physical activity appears to be a key determinant of the prevalence of central obesity in this study. Preventive actions such as exercise and active lifestyles have to be implemented to reduce the tendency for central obesity in this population in particular and the general Ghanaian populace at large.
Journal of Medical and Biomedical Sciences (2018) 7(2), 1-9

Keywords*:* WHR, Civil Servants, Physical Activity, Diets, Tamale

INTRODUCTION

There is a worldwide obesity epidemic with the prevalence of overweight and obesity exceeding 50% in almost all the regions of the world (Balkau *et al.*, 2007). Over the past three decades, this epidemic has affected the industrialized countries, with some areas of North America, Europe and Asia having more than threefold increase in the prevalence of obesity (Fezeu *et al.*, 2008; Parikh *et al.*, 2007; Wang *et al.*, 2007). In recent times, low- and medium-income countries have joined the obesity epidemic, and the increase has been faster in these countries (Fezeu *et al.*, 2008; Wang *et al.*, 2007).

Genetics and lifestyle changes such as diet and physical activity level can greatly modify the prevalence and severity of obesity in adults (Helge, 2002). In a healthy adult population, there is an inverse relationship between the level of physical activity and obesity (Ford *et al.*, 2002; Katzmarzyk *et al.*, 2004; Owiredu *et al.*, 2011). The causes and prevalence of obesity vary from population to population based on individual life style, diet, cultural background, genetic make-up as well as the type of instrument used in the assessment of obesity.

Correspondence: *Mr. V. Mogre, Department of Human Biology, School of Medicine and Health Sciences, University for Development Studies, Tamale, Ghana. E*-mail: mogrevictor@yahoo.com

The prevalence of obesity was found to be 5.5% in Ghana and higher among women (7.4%) compared to men (2.8%) using body mass index (Biritwum *et al.*, 2005). In a study conducted by Owiredu *et al.,* (2011) in Kumasi, the prevalence of obesity was found to be higher among sedentary workers (17.4%) compared to active subjects (5.9%) using body mass index. On the other hand, using waist to hip ratio, the prevalence of central obesity was found to be 27.9% among sedentary workers as compared to 2.9% in active subjects (Owiredu *et al.*, 2011). In Ghana there is paucity of data on the prevalence of central obesity among civil servants. However, with the increasing westernization of Ghanaian lifestyle and eating habits the prevalence of obesity has been reported to be on the increase across the country. This study therefore sought to find the prevalence of central obesity among civil servants in the Tamale Metropolis as well as the impact of diet and physical activity level.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted between January and July, 2011. The subjects comprised of 186 adult civil servants in the Tamale Metropolis in the Northern Region of Ghana. All personnel from the 13 civil service departments in the Tamale Metropolis were eligible to participate in the study. The subjects were selected using a proportionate random sample that included more subjects from larger departments with the use of a random numbers statistical table. The participation of the subjects was voluntary and informed consent was obtained from each subject. Subjects who were on medication known to modify serum lipid or carbohydrate metabolism were excluded from the study. The study was approved by the Ethics Committee of the University for Development Studies, School of Medicine and Health Sciences, Ghana.

Anthropometric variables

Anthropometric measurements included waist circumference, measured midway between the inferior angle of the ribs and the suprailiac crest. Hip circumference, measured as the maximal circumference over the buttocks in centimetres. Both measurements were measured to the nearest 1 cm using a non-stretchable fibre-glass measuring tape (Butterfly, China). During both measurements, subjects were made to stand upright, with arms relaxed at the side, feet evenly spread apart and body weight evenly distributed in accordance with the WHO expert consultation report on waist circumference and waist-hip ratio (WHO, 2004). Waist to Hip Ratio (WHR) was calculated by dividing the waist circumference (cm) by the hip circumference (cm). Men with WHR < 0.90, 0.90–0.99 and ≥ 1.0 were classified as normal, overweight or obese respectively, while women were classified in the same categories on the basis of WHR of < 0.80, 0.80 – 0.84 and ≥ 0.85 (WHO, 2004).

Physical activity level

The level of physical activity was assessed using the Global Physical Activity Questionnaire (GPAQ) (WHO, 2009). The GPAQ comprised of 19 questions about physical activity level in a typical week. The frequency and duration of time spent doing physical activity is measured in 3 domains: activity at work, travel to and from places and recreational activities.

The GPAQ was selected as a result of its standardization, easiness to administer, relative unobtrusiveness and inexpensiveness. Its reliability has been found to be 0.67–0.81 and the validity was 0.54 (Armstrong *et al.*, 2006). The questionnaire was fully adapted for the study, without any modifications. However, to suit the Ghanaian context, local examples of types and intensity of activities were used. The collection of all data and processing followed the GPAQ analysis protocol (WHO, 2009). All durations were converted into minutes. Energy expenditure, measured in MET (Metabolic equivalents), was estimated using duration, intensity and frequency of physical activities performed within 7-days. MET, is the ratio of specific physical activity metabolic rates to the resting metabolic rate (1 MET = the energy cost of sitting quietly, and was equivalent to a caloric consumption of 1 kcal/kg/hour). A MET-minute showed the total activity volume on weekly basis, and calculated by multiplying time spent on each activity during a week by the

MET-values of each level of activity. Using the compendium of physical activities (Ainsworth *et al.*, 2000), MET-values for various levels of activities was established. MET values of 4 and 8 were set for moderate-intensity (transport-related walking or cycling) and vigorous-intensity physical activity, respectively. Total MET/minutes/week was computed by the sum of all moderate- to vigorous-intensity physical activities performed at work, transport and recreation. Based on the total met/minutes/week, subjects were classified into low, moderate, and high physical activity levels as defined by the GPAQ analysis framework (WHO, 2009).

High: A subject found within any of the following categories: Vigorous-intensity activity on at least three days achieving at least 1,500 MET-minutes/ week OR seven or more days of any combination of walking, moderate or vigorous intensity activities achieving at least 3,000 MET-minutes per week.
Moderate: A subject not achieving the criteria for the high category but either of the following three criteria: (a) 3 or more days of vigorous-intensity of at least 20 minutes per day OR (b) 5 or more days of moderate-intensity and/ or walking of at least 30 minutes per day OR (c) 5 or more days of any combination of walking, moderate-or vigorous- intensity activities accumulating at least 600 MET minutes/ week.
Low: Subject's reported activity is lower than the categories outlined above or no activity is reported at all.

Dietary intake
A validated FFQ was used for assessing usual dietary intakes (Ovaskainen *et al.*, 2006; Rolls *et al.*, 2006). The FFQ was adapted and modified from the Willett food frequency questionnaire in which 38 food items were listed (Willett *et al.*, 1987). FFQs have been shown to be valuable tools for evaluating long-term dietary intake, especially in the context of epidemiological studies such as this (Baer *et al.*, 2005). The questionnaire considered the number of times subjects ate some listed food items. The listed food items were put into six food groups: carbohydrates (e.g. rice, tuo zaafi (T.Z), etc), proteins (animal source: meat, beans, fish, and plant source: beans, nuts, etc), fats and oils (vegetable oil, palm oil, etc), fruits and vegetables (pineapple, mango, etc), non-alcoholic beverages (soft drinks, etc) and alcoholic drinks (beer, whisky, wine, etc).

Statistical Analysis
The results are expressed as proportion and compared using Fischer's exact test or χ^2 for trend analysis as appropriate. A level of $p<0.05$ was considered as statistically significant. GraphPad Prism version 5.00 (GraphPad software, San DiegoCalifornia USA, www.graphpad.com) for windows was used for statistical analysis.

RESULTS
The general characteristics of the studied population are as shown in Table 1. Majority of the studied population were within 20 to 39 years i.e. 150/186 (80.6%) and have attained a high level of education (89.8%). Even though, male participants represent 65.1% of the studied population, there is no significant difference in the age distribution and educational level between the male and female subjects (Table 1). From this study, the prevalence of obesity and overweight as determined by WHR was 31.2% and 12.4% respectively. When the study population was stratified based on gender, 64.5% of the male subjects significantly ($p = 0.0004$) had normal body weight compared to female subjects (41.5%). However, the prevalence of obesity was significantly ($p = 0.0312$) higher among the female participants (41.5%) compared to males (25.6%) (Table 1).

Whereas about 10% of the studied population was engaged in high level of physical activity, about 30% were engaged in moderate level of physical activity and about 60% were engaged in low level of physical activity as shown in Table 1. There was no significant difference in the levels of physical activity when the studied population was classified by gender. About 27%, 44%, 10% and 5% of the studied population consumed at least one form of carbohydrate rich foods, protein rich foods of animal source, protein rich foods of plant source as well as fats and oils rich foods respectively for more than once a day. Gender had no significant impact on the type of food groups consumed by the studied population (Table 1).

Table 1: General characteristic of the study population stratified by gender

Variable	Total (n=186)	Male (n=121)	Female (n=65)	P value
Age				
20-29	73(39.2%)	43(35.5%)	30(46.2%)	0.161
30-39	77(41.4%)	51(42.1%)	26(40.0%)	0.876
40-49	17(9.1%)	13(10.7%)	4(6.2%)	0.425
50-59	19(10.2%)	14(11.6%)	5(7.7%)	0.458
Educational level				
High	167(89.8%)	108(89.1%)	59(90.8%)	0.805
Central adiposity				
Normal	105(56.4%)	78(64.5%)	27(41.5%)	0.001
Overweight	23(12.4%)	11(9.1%)	12(8.5%)	0.099
Obese	58(31.2%)	31(25.6%)	27(41.5%)	0.031
Physical activity level				
Low	106(57.0%)	68(56.2%)	38(58.5%)	0.876
Moderate	60(32.3%)	39(32.2%)	21(32.3%)	1.000
High	20(10.8%)	14(7.5%)	6(9.5%)	0.805
Diet frequency				
Carbohydrate-rich foods	50(26.9%)	34(28.1%)	16(24.6%)	0.729
Animal protein	82(44.1%)	54(44.6%)	28(43.1%)	0.839
Plant protein	18(9.7%)	11(9.1%)	7(10.8%)	0.712
Fats & Oils	9(4.8%)	5(4.1%)	4(6.2%)	0.540
Fruits & vegetables	29(15.6%)	17(14.0%)	12(18.5%)	0.429
Alcoholic beverages	4(2.2%)	4(3.3%)	0(0.0%)	0.138
Non-Alcoholic beverages	26(14.0%)	15(12.4%)	11(16.9%)	0.396

Data are presented as proportion and analyzed using Fischer's exact test.

From Table 2, using chi-square for trend analysis, there is no significant association between central obesity as determined by WHR and food groups consumed by the studied population using chi square for trend analysis. In Table 3, the type of food groups consumed was stratified based on age. The proportion of the studied population that consumed at least one form of carbohydrate rich food, protein rich food of animal source, fats and oils rich food as well as those that consumed fruits and vegetables for more than once a day generally decreased with age (Table 3).

Table 2: Diet frequency and central adiposity

Variable	Normal (n=105)	Overweight (n=23)	Obese (n=58)	P value
Carbohydrate-rich foods	32(30.5%)	4(17.4%)	14(24.1%)	0.324
Animal protein	46(43.8%)	7(30.4%)	29(50.0%)	0.542
Plant protein	12(11.4%)	0(0.0%)	6(10.3%)	0.689
Fats & Oils	6(5.7%)	0(0.0%)	3(5.2%)	0.783
Fruits & vegetables	16(15.2%)	3(13.0%)	10(17.2%)	0.765
Alcoholic beverages	2(1.9%)	0(0.0%)	2(3.4%)	0.570
Non-Alcoholic beverages	15(14.3%)	4(17.4%)	7(12.1%)	0.737

Table 3: The rate of consumption of the various diet group stratified by age

Variable	20-29 (n=73)	30-39 (n=77)	40-49 (n=17)	50-59 (n=19)	P value
Carbohydrate-rich	26(35.6%)	21(27.3%)	2(11.8%)	1(5.3%)	0.002
Animal protein	44(60.3%)	31(40.3%)	3(17.6%)	4(21.1%)	0.001
Plant protein	7(9.6%)	8(10.4%)	1(5.9%)	2(10.5%)	0.945
Fats & Oils	7(9.6%)	2(2.6%)	0(0.0%)	0(0.0%)	0.025
Fruits & vegetables	17(23.3%)	10(13.0%)	1(5.9%)	1(5.3%)	0.016
Alcoholic beverages	2(2.7%)	2(2.6%)	0(0.0%)	0(0.0%)	0.385
Non-alcoholic beverages	13(17.8%)	9(11.7%)	2(11.8%)	2(10.5%)	0.313

Data was presented as proportion and analyzed using chi-square for trend

The association between age and physical activity level with body weight is presented in figure 1. From the chi-square for trend analysis, the prevalence of obesity significantly increased with age ($p < 0.001$) but decreased with level of physical activity ($p = 0.018$).

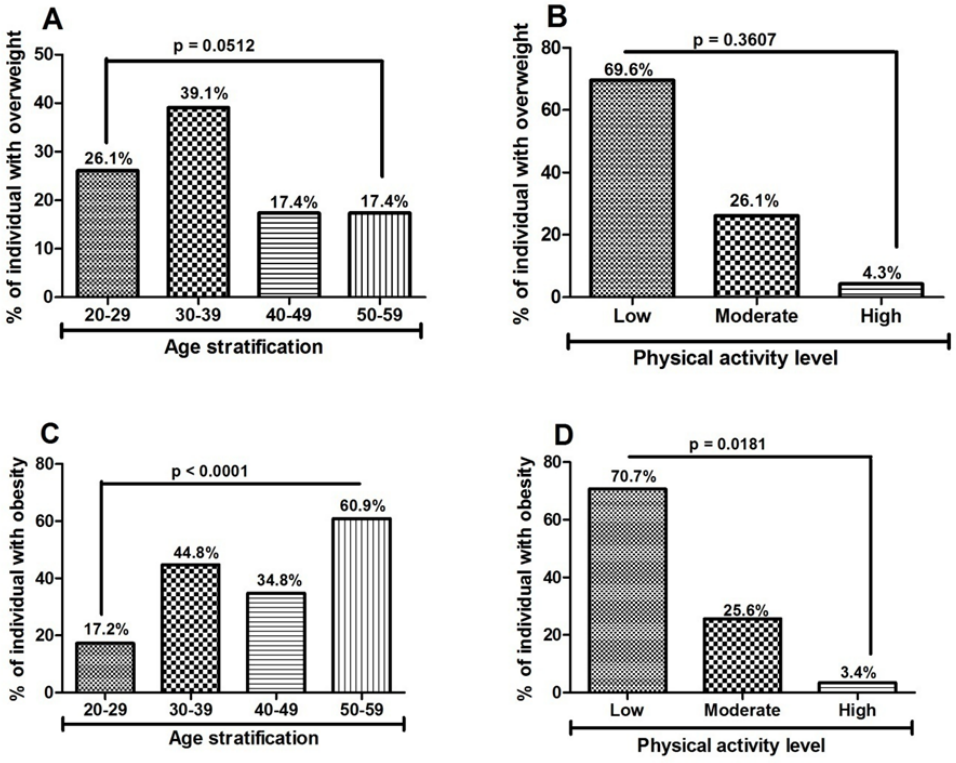


Figure 1: Association between age and overweight (**A**), physical activity level and overweight (**B**), age and obesity (**C**) as well as physical activity level and obesity (**D**). Data was analyzed using chi-square for trend.

DISCUSSION

From the study, the overall prevalence of central obesity was found to be 31.2%. This is consistent with a central obesity prevalence of 31.9% reported in a study among an industrial population in India (Reddy *et al*., 2006). The prevalence of central obesity in this study was higher than the 20% reported by Addo *et al.,* (2009) among civil servants in Accra and the 14.4% (defined by WHR) reported by Owiredu *et al.,* (2011) in Kumasi. Addo *et al.,* (2009) used Body Mass Index (BMI) to define obesity and this could have accounted for the difference in prevalence. Ferland *et al.,* (1989) reported that WHR was a good predictor of intra-abdominal adipose tissue than BMI. Furthermore, BMI does not account for the wide variation in body fat distribution and has considerable limitations in predicting intra-abdominal fat accumulation (Chen *et al*., 2000). BMI is therefore not able to differentiate between subcutaneous from visceral fat accumulation (Fujimoto *et al*., 1999). On the other hand, WHR provides an index of both subcutaneous and intra-abdominal adipose tissue (Bjorntorp, 1987), making the central obesity rates defined by it being higher than rates measured by BMI. Owiredu *et al.,* (2011) conducted their study among active sportsmen/sportswomen and sedentary workers and this could have contributed to the lower prevalence of central obesity. The subjects in that study included active sportsmen and women, who were generally physically active. Subjects in this research however had lower levels of physical activity demonstrated by the inverse relationship between the level of physical activity and central obesity observed in this study.

Central obesity was significantly higher in women than men and this significant finding is in agreement with several studies done elsewhere in Ghana (Hill *et al*., 2007) and in other African countries (Maher *et al*., 2011; Shayo *et al*., 2011; Wahab *et al*., 2011). Participation in regular physical activity was generally low in this study and was even lower among females. This fact, coupled with the general perception among African women that being obese is a sign of affluence perhaps accounts for such a high obesity prevalence rate among women involved in this study. In a study by Ojofeitimi and colleagues in south western Nigeria, subjects in a University community believed that being obese is a sign of well-being and gives respect (Ojofeitimi *et al*., 2007).

Generally, obesity increased significantly with increase in age category in this study. These findings are in consonance with prevalence of central obesity found among senior civil servants in Nigeria (Odenigbo *et al*., 2008), South-African adults in South Africa (Puoane *et al*., 2002) and Cuban adults in Cuba (Diaz *et al*., 2009). The proportion of fat deposited in the abdomen increases as body shape becomes more android with age, due to decreasing height and increasing slackness of abdominal wall muscles. During adulthood, weight gain occurs in the abdominal region, emphasizing the importance of hypertrophic obesity, which is generally android (Kaye *et al*., 1990). This change in the adult figure may influence the positive association between age and excess abdominal adiposity, measured by waist-to-hip ratio (Lanska *et al*., 1985). This fact possibly explains the increase in obesity prevalence with increasing age category in this study.

In a study on the association of body fat distribution with lifestyle and reproductive factors in post-menopausal women, Kaye *et al.,* (1990) showed that waist-to-hip ratio was negatively associated with physical activity. In addition, a study of subjects from two urban and one rural community in Accra, Ghana, found high levels of overweight and obesity among subjects who were sedentary and engaged in light activity (Amoah, 2003). These findings are consistent with the findings of this current study which showed that subjects with low physical activity levels had significantly high prevalence of overweight (69.6%) and central obesity (70.7%) and the prevalence of both overweight and obesity decreased with increase in the level of physical activity. Similarly, a population based cross-sectional study conducted by Al-Nozha and colleagues among Saudis aged 30-70 years, reported that inactive males and females had a significantly higher waist circumference than active participants of both sexes (Al-Nozha *et al*., 2007). In a study, in which CT scan was used to assess abdominal obesity, physical activity was shown to be strongly associated with lower

visceral adipose tissue in men from 30-70 years (Hunter *et al.*, 1997). One can therefore not overemphasize the well-established protective role of physical activity for the development of obesity and associated co-morbidities. The role of physical activity in the prevention and management of overweight and obesity is linked, in part, to the impact of physical activity on energy expenditure, body composition, and substrate oxidation and metabolism (Donnelly *et al.*, 2004). Policies that would help individuals build a positive attitude towards exercise should be instituted so as to cultivate the habit of engaging in regular and beneficial physical exercises.

There was no significant association between dietary intake and obesity. As indicated in the results of this study, subjects consumed less of both high energy dense foods and fruits and vegetables. This suggests that the high prevalence rate of central obesity found in this research cannot be attributed to dietary intake, but could be due to low levels of physical activity and age. In contrast to the works of other researchers, no relationship was found between alcohol and obesity in this study (Breslow *et al.*, 2005; Colditz *et al.*, 1991; Schroder *et al.*, 2007; Wannamethee *et al.*, 2003). The lack of association could be explained by the small proportion of study participants who indulged in alcohol drinking. The small proportion of alcohol drinkers could be due to the fact that a greater majority of the inhabitants in the Tamale metropolis are moslems (Ghana Government Official Portal, 2012) and as required by the Islamic faith, do not consume alcoholic beverages of any kind.

CONCLUSION

Prevalence of central obesity is high in this study. In consonance with most studies, female subjects were more at risk compared to their male counterparts. Level of physical activity and age category were major determinants of central obesity. Individuals should be encouraged to engage in regular physical exercise.

REFERENCES

Addo J, Smeeth L, Leon DA (2009) Obesity in urban civil servants in Ghana: association with pre-adult wealth and adult socio-economic status. *Public health* **123**(5)**:** 365-370.

Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DRJ, Schmitz KH, Emplaincourt PO, Jacobs DRJ, Leon AS (2000) Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc.* **32**(9)**:** S498-504.

Al-Nozha MM, Al-Hazzaa HM, Arafah MR, Al-Khadra A, Al-Mazrou YY, Al-Maatouq MA, Khan NB, Al-Marzouki K, Al-Harthi SS, Abdullah M, Al-Shahid MS (2007) Prevalence of physical activity and inactivity among Saudis aged 30-70 years. A population -based cross-sectional study. *Saudi medical journal* **28**(4)**:** 559-568.

Amoah AG (2003) Sociodemographic variations in obesity among Ghanaian adults. *Public health nutrition* **6**(8)**:** 751-757.

Armstrong T, Bull F (2006) Development of the World Health Organization Global Physical Activity Questionnaire (GPAQ). *J Public Health (Oxf)* **14:** 66-70.

Baer H, Blum R, Rockett H, Leppert J, Gardner J, Suitor C, Colditz G (2005) Use of a food frequency questionnaire in American Indian and Caucasian pregnant women: a validation study. *BMC public health* **5**(1)**:** 135.

Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox KA, Smith SC, Jr., Barter P, Tan CE, Van Gaal L, Wittchen HU, Massien C, Haffner SM (2007) International Day for the Evaluation of Abdominal Obesity (IDEA): a study of waist circumference, cardiovascular disease, and diabetes mellitus in 168,000 primary care patients in 63 countries. *Circulation* **116**(17)**:** 1942-1951.

Biritwum R, Gyapong J, Mensah G (2005) The epidemiology of obesity in ghana. *Ghana Med J* **39**(3)**:** 82-85.

Bjorntorp P (1987) Fat cell distribution and metabolism. *Ann N Y Acad Sci* **499:** 66-72.

Breslow RA, Smothers BA (2005) Drinking patterns and body mass index in never smokers: National Health Interview Survey, 1997-

2001. *American journal of epidemiology* **161**(4)**:** 368 -376.

Chen CH, Lin KC, Tsai ST, Chou P (2000) Different association of hypertension and insulin-related metabolic syndrome between men and women in 8437 nondiabetic Chinese. *Am J Hypertens* **13**(7)**:** 846-853.

Colditz GA, Giovannucci E, Rimm EB, Stampfer MJ, Rosner B, Speizer FE, Gordis E, Willett WC (1991) Alcohol intake in relation to diet and obesity in women and men. *The American journal of clinical nutrition* **54**(1)**:** 49-55.

Diaz ME, Jimenez S, Garcia RG, Bonet M, Wong I (2009) Overweight, obesity, central adiposity and associated chronic diseases in cuban adults. *MEDICC Rev* **11**(4)**:** 23-28.

Donnelly JE, Smith B, Jacobsen DJ, Kirk E, Dubose K, Hyder M, Bailey B, Washburn R (2004) The role of exercise for weight loss and maintenance. *Best Pract Res Clin Gastroenterol* **18** (6)**:** 1009-1029.

Ferland M, Despres JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, Lupien PJ, Theriault G, Bouchard C (1989) Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br J Nutr* **61**(2)**:** 139-148.

Fezeu LK, Assah FK, Balkau B, Mbanya DS, Kengne AP, Awah PK, Mbanya JC (2008) Ten-year changes in central obesity and BMI in rural and urban Cameroon. *Obesity (Silver Spring, Md* **16**(5)**:** 1144-1147.

Ford ES, Giles WH, Dietz WH (2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* **287**(3)**:** 356-359.

Fujimoto WY, Bergstrom RW, Boyko EJ, Chen KW, Leonetti DL, Newell-Morris L, Shofer JB, Wahl PW (1999) Visceral adiposity and incident coronary heart disease in Japanese-American men. The 10-year follow-up results of the Seattle Japanese-American Community Diabetes Study. *Diabetes Care* **22**(11)**:** 1808-1812.

Ghana Government Official Portal (2012) About Ghana: Northern Region.

Helge J (2002) Prolonged adaptation to fat-rich diet and training; effects on body fat stores and insulin resistance in man. *Intern J Obes* **26:** 1118-1124.

Hill AG, Darko R, Seffah J, Adanu RM, Anarfi JK, Duda RB (2007) Health of urban Ghanaian women as identified by the Women's Health Study of Accra. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics* **99**(2)**:** 150-156.

Hunter GR, Kekes-Szabo T, Snyder SW, Nicholson C, Nyikos I, Berland L (1997) Fat distribution, physical activity, and cardiovascular risk factors. *Medicine and science in sports and exercise* **29**(3)**:** 362-369.

Katzmarzyk PT, Church TS, Blair SN (2004) Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men. *Arch Intern Med* **164**(10)**:** 1092-1097.

Kaye SA, Folsom AR, Prineas RJ, Potter JD, Gapstur SM (1990) The association of body fat distribution with lifestyle and reproductive factors in a population study of postmenopausal women. *Int J Obes* **14**(7)**:** 583-591.

Lanska DJ, Lanska MJ, Hartz AJ, Rimm AA (1985) Factors influencing anatomic location of fat tissue in 52,953 women. *Int J Obes* **9**(1)**:** 29-38.

Maher D, Waswa L, Baisley K, Karabarinde A, Unwin N, Grosskurth H (2011) Distribution of hyperglycaemia and related cardiovascular disease risk factors in low-income countries: a cross-sectional population-based survey in rural Uganda. *Int. J. Epidmiol.* **40**(1)**:** 160-171.

Odenigbo CU, Oguejiofor OC, Odenigbo UM (2008) Prevalence of abdominal obesity in senior civil servants in Asaba, Nigeria. *Tropical Journal of Medical Research* **12**(2)**:** 1-3.

Oh JY, Hong YS, Sung YA, Barrett-Connor E (2004) Prevalence and factor analysis of metabolic syndrome in an urban Korean population. *Diabetes care* **27**(8)**:** 2027-2032.

Ojofeitimi EO, Adeyeye AO, Fadiora AO, Kuteyi AO, Faborode TG, Adegbenro CA, Bakare

OE, Setiloane K, Towobola KS (2007) Awareness of obesity and its health hazard among women in a university community. *Pakistan Journal of Nutrition* **6**(5)**:** 502-505.

Ovaskainen ML, Reinivuo H, Tapanainen H, Hannila ML, Korhonen T, Pakkala H (2006) Snacks as an element of energy intake and food consumption. *European journal of clinical nutrition* **60**(4)**:** 494-501.

Owiredu WKBA, Amidu N, Gockah-Adapoe E, Ephraim RKD (2011) The prevalence of metabolic syndrome among active sports-sportswomen and sedentary workers in the kumasi metropolis. *Journal of Science and Technology* **31**(1)**:** 23-36.

Parikh NI, Pencina MJ, Wang TJ, Lanier KJ, Fox CS, D'Agostino RB, Vasan RS (2007) Increasing trends in incidence of overweight and obesity over 5 decades. *The American journal of medicine* **120**(3)**:** 242-250.

Puoane T, Steyn K, Bradshaw D, Laubscher R, Fourie J, Lambert V, Mbananga N (2002) Obesity in South Africa: the South African demographic and health survey. *Obes Res* **10** (10)**:** 1038-1048.

Reddy KS, Prabhakaran D, Chaturvedi V, Jeemon P, Thankappan KR, Ramakrishnan L, Mohan BV, Pandav CS, Ahmed FU, Joshi PP, Meera R, Amin RB, Ahuja RC, Das MS, Jaison TM (2006) Methods for establishing a surveillance system for cardiovascular diseases in Indian industrial populations. *Bull World Health Organ* **84**(6)**:** 461-469.

Rolls BJ, Roe LS, Meengs JS (2006) Reductions in portion size and energy density of foods are additive and lead to sustained decreases in energy intake. *The American journal of clinical nutrition* **83**(1)**:** 11-17.

Schroder H, Morales-Molina JA, Bermejo S, Barral D, Mandoli ES, Grau M, Guxens M, de Jaime Gil E, Alvarez MD, Marrugat J (2007) Relationship of abdominal obesity with alcohol consumption at population scale. *Eur J Nutr* **46**(7)**:** 369-376.

Shayo GA, Mugusi FM (2011) Prevalence of obesity and associated risk factors among adults in Kinondoni municipal district, Dar es Salaam Tanzania. *BMC public health* **11**(1)**:** 365.

Wahab KW, Sani MU, Yusuf BO, Gbadamosi M, Gbadamosi, A, Yandutse, MI (2011) Prevalence and determinants of obesity - a cross-sectional study of an adult Northern Nigerian population. *International Archives of Medicine* **4**(1)**:** 10.

Wang H, Du S, Zhai F, Popkin BM (2007) Trends in the distribution of body mass index among Chinese adults, aged 20-45 years (1989-2000). *International journal of obesity (2005)* **31**(2)**:** 272-278.

Wannamethee SG, Shaper AG (2003) Alcohol, body weight, and weight gain in middle-aged men. *The American journal of clinical nutrition* **77** (5)**:** 1312-1317.

WHO (2004) Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* **363** (9403)**:** 157-163.

WHO (2009) Global Physical Activity Surveillance. Available from: http://www.who.int/chp/steps/GPAQ/en/index.html; [cited 2 April 2009].

Willett WC, Reynolds RD, Cottrell-Hoehner S, Sampson L, Browne ML (1987) Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. *Journal of the American Dietetic Association* **87**(1)**:** 43-47.

ISSN 2026-6294

Journal of Medical and Biomedical Sciences (2018) 7(2): 10 -16

ORIGINAL ARTICLE

Haematopoietic effect of an ethanolic leaf extract of *Ipomoea involucrata* P. Beauv in phlebotomized New Zealand White Rabbits

G.A. Koffuor[1], P.E. Dadzeasah[1] and G.H. Sam[2]

[1]Department of Pharmacology, [2]Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana

Ipomoea involucrata **is used by some religious bodies (that do not believe in the use of allopathic medicine) in Ghana to treat haematological conditions particularly anaemia. The aim of this study therefore is to determine the haematopoietic effect of the ethanolic leaf extract of *Ipomoea involucrata* to establish the scientific bases for its use. The haematological profile of healthy New Zealand rabbits, phlebotomized rabbits, and phlebotomized rabbits treated with 0.23 ml/kg Feroglobin®, 100, 300, and 1000 mg/kg *I. involucrata* ethanolic leaf extract, or 0.23 ml normal saline were determined at 20-day intervals for 40 days using the Cell Dyn 1800 Automatic Analyzer®. Data obtained was analyzed using GraphPad Prism version 5. The 300 and 1000 mg/kg dose of the extract and the reference hematinic caused significant increments ($P \leq 0.01$ -0.001) in white blood cells, red blood cells, haemoglobin concentration, hematocrit, and platelets counts within 20 days of treatment. The mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration, and the red cell distribution width recorded in all categories were not significantly different. This indicates that the ethanolic leaf extract of *Ipomoea involucrat*a has some haematopoietic activity and thus could be effective in managing anaemia and other blood cell deficiency disorders.**

Journal of Medical and Biomedical Sciences (2018) 7(2), 10-16

Keywords*:* Phlebotomy, Haemoglobin, RBC, Haematological Profile

INTRODUCTION

Anaemia, the most predominant blood cell deficiency disorder, is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development (de Benoist *et al.,* 2008). It occurs at all stages of life, but is more prevalent in pregnant women and young children. More frequently it coexists with a number of other causes, such as malaria, parasitic infection, nutritional deficiencies, and haemoglobinopathies (de Benoist *et al.,* 2008). Other blood cell deficiency disorders are neutropenia and thrombocytopenia.

Correspondence: *Mr. G.A. Koffuor, Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. E*-mail: gkoffuor@yahoo.com

Severe anaemia (prevalence exceeds 2%) is a problem in most countries in Africa and South Asia and some countries in East Asia and the Pacific (Galloway, 2003; Stoltzfus, 2003). In most parts of Africa the main causes of anaemia are usually due to poor nutrition and malaria. An earlier study has shown prevalence of anaemia in southern Ghana to be fairly common, particularly in children and women (Commey and Dekyem, 1995).

Low income levels in Ghana (Obeng, 2008) pose a challenge in the affordability of orthodox haematinics. Access to health centers in rural areas is also a problem; meanwhile as much as 80 % of people in developing countries are said to depend on traditional medicines for primary healthcare (Bodekar, 1994; WHO, 2008). In fact, the Alma Ata declara-

tion of 1978 encouraged the use of all available resources for primary healthcare and recommended that government should give high priority to using traditional health practices and incorporate proven traditional remedies into National Drug Policy and Regulations (Zhang, 1994). One widely used traditional remedy for anaemia is *Ipomoea involucrata*.

Ipomoea involucrata P. Beauv.*var. involucrata* (Family: Convolvulaceae) commonly known as Dutchman's pipe is a slender but vigorous, sprawling or twining annual or perennial herb, of grassland, secondary scrub and forest origin very common throughout tropical Africa. Its several medicinal uses are as follows: an infusion of the whole plant is drunk as a stimulant, or preventative of fever; a decoction of the fresh sap is taken as a remedy for gonorrhoea; the leaves are used for asthma (Oliver, 1960); a plant preparation is added to baths or made into a lotion for treating jaundice (Bouquet & Debray, 1974); the leaf-sap is applied and rubbed into areas of localized edema and is instilled into the eyes for filarial infection; an aqueous decoction is taken by women for dysmenorrhea and at child-birth to hasten expulsion of the after-birth; and a compress of pounded up stems is used for headache (Bouquet, 1969).

It is in this light that this study has been conducted to determine the haematopoietic effect of *Ipomoea involucrata* to establish the scientific bases for its use and to add to the armamentarium of herbs that can be used to manage anaemia in Ghana.

MATERIALS AND METHODS

Plant Collection

Ipomoea involucrata was collected from KNUST campus in August 2008 and authenticated by Mr. G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana where a voucher specimen with number KNUST/HM1/2011/R004 has been deposited. The leaves were harvested, air dried, and powdered using a hammer mill (Schutte Buffalo, New York, USA).

Preparation of Extract

A 220 g quantity of the leaf powder of *Ipomoea involucrata* was soxlet extracted with 70 % ethanol. The liquid extract obtained was condensed under low temperature and pressure using a Buchi Rotor Evaporator (Rotavapor R-210, Switzerland), and further dried over a hot water bath (B-Tex Laboratory Engineering, Gujarat India) at 40 ^{o}C. The dried extract weighing 18.5 g (percentage yield: 8.4) was labeled and stored in a desiccator. Required quantities taken and dissolved in distilled water for use in this study will be referred to as the ethanolic extract of *Ipomoea involucrata* or EIE.

Phytochemical Screening

The ethanolic leaf extract of *Ipomoea involucrata* was subjected to phytochemical screening in accordance with the standard procedure (Harborne 1998).

Animals and Husbandry

Male New Zealand White Rabbits (4.8 – 5.2 kg) obtained from Kwadaso Agricultural College, Kumasi, Ghana were kept in the Department of Pharmacology, KNUST, animal house for this study. They were fed with normal rabbit chow (GHAFCO, Tema, Ghana), fresh herbs like *Aspilia Africana* and rough meadow-grass (*Poa trivialis*) from around the KNUST campus, and clean water. During the experimental period, the rabbits were kept in clean, well-ventilated, individual, front-opening stainless steel cages (that had grid plastic floor) in the laboratory under ambient dark-light cycle and relative humidity. The rabbits were allowed to acclimatize to the laboratory environment for one week prior to experimentation. During this period, the animals were periodically assessed for gross morphological and behavioral changes.

All procedures and techniques used in these studies were in accordance with 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). The protocols for the study were approved by the Departmental Ethics Committee.

Reference Haematinic

Feroglobin® (Vitabiotics Ltd, Great Britain), a liquid tonic containing Iron 0.2 %, Zinc 0.06 %, Cop-

per 0.02 %, Manganese 0.025 %, and vitamin B-Complex 0.39 % in a blend of honey and malt, was used as the reference hematinic. The dose of Feroglobin® administered to experimental animals in this study is equivalent to the adult human dose (0.23 ml/kg/day) stated by the manufacturers.

Phlebotomization of Rabbits

Phlebotomization of the New Zealand White rabbits was achieved by following a rabbit bleeding protocol (McGuill and Rowan, 1989) after their basal haematological profile had been determined using the Abbot Cell Dyn 1800 Automatic Analyzer® (Abbott Diagnostics, USA). In this protocol, the rabbits were sedated using sodium thiopentone (60 mg/kg, i.p). The left ears were shaved to expose the auricular artery (which runs medially in the ear). This was disinfected with 70% ethanol and rubbed gently with 30% limonene in 95% ethanol to dilate it. A hypodermic needle (22, or 20 gauge, 1 inch long) attached to a calibrated tube was inserted into the central artery about two-thirds the way up the central artery, with the tip of the needle pointing towards the base of the ear (McGuill and Rowan, 1989). About 15-20 % of the total volume of blood, calculated based on 60 ml/kg per rabbit (Suckow *et al.,* 2002), in the rabbit were collected into the calibrated tube. When blood collection was completed, the needle was removed and the artery held off firmly with cotton wool for 3 minutes until the bleeding stopped. Drops and smears of blood were cleaned with 70% ethanol to prevent infection of punctured site. The rabbits were returned to the cage when there was no further bleeding which is indicative of restored circulation. Phlebotomization was considered satisfactory and normocytic anaemia was considered induced when blood cell counts reduced by at least 30% after the haematological profile were determined after 24 hours.

Experimental Procedure

The phlebotomized rabbits were weighed and put into five groups (A-E) with five animals per group for the study. The groups were treated with 0.23 ml/kg Feroglobin®, 100, 300, or 1000 mg/kg EIE, or 0.23 ml/kg normal saline for 40 days. A sixth group, F, with 5 healthy rabbits was also kept under the laboratory conditions but these were not given any form of treatment. At day 20 and day 40, blood samples from animals in all groups were collected into MediPlus K3 EDTA tubes (Sunphoria Co. Ltd., Taiwan) for haematological analysis using the Abbot Cell Dyn 1800 Automatic Analyzer®. Data obtained on white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), and platelet count (PLT) were recorded for analysis.

Statistical Analysis

Significant changes in measured parameters between normal, phlebotomized, and phlebotomized but treated rabbits were determined using One-Way Analysis of Variance followed by Dunnet's Multiple Comparison Test. Statistical estimates were made at a confidence limit of 95% and probability values ($P \leq 0.05$ were considered significant. The analysis was done using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Phytochemical Screening

Results from the phytochemical screening of EIE is as shown in Table 1.

Table 1: Results obtained from phytochemical screening of ethanolic extract of *Ipomoea involucrate* (EIE)

Components	EIE
Tannins	+
Flavonoids	-
Alkaloids	+
Sterols	-
Glycosides	+
Saponins	+
Triterpenoids	-

(+) indicates the presence of; (-) indicates the absence of the phytochemicals tested

Phlebotomization

There was a very significant reduction ($P \leq 0.001$) in RBCs, HGB, HCT and PLT after phlebotomy. The WBC was also significantly ($P \leq 0.01$) reduced; however, there were insignificant changes ($P > 0.05$) in MCV, MCH, MCHC, and RDW (Table 2).

Haematopoietic effect

Treatment of phlebotomized rabbits with 300 mg/kg EIE for 20 days resulted in significant increments ($P \leq 0.05$) in WBC, RBCs, and very significant increments ($P \leq 0.001$) in HGB, HCT and PLT. The 1000 mg/kg EIE also caused significant increments ($P \leq 0.05$) in WBC and very significant increments ($P \leq 0.01$) in RBC and PLT. HGB, and HCT were also very significantly ($P \leq 0.001$) elevated. The reference hematinic showed very significant elevation ($P \leq 0.01$) in WBC, RBC, and PLT as well as very significant elevation ($P \leq 0.001$) in HGB and HCT. Changes in MCV, MCH, MCHC, and RDW with treatments were however insignificant (Table 3). After 40 days of treatment, the reference drug-treated and the 1000 mg/kg-treated groups showed very significant increments ($P \leq 0.01$) in WBC, and even more significant increments ($P \leq 0.001$) in RBCs, HGB, HCT and PLT. The 100 mg/kg EIE treatment also showed some significant increments ($P \leq 0.05$) in WBC and PLT and also significant increments in RBC, HGB, and HCT (not seen with the normal saline-treated group). With the exception of WBC which increased significantly ($P \leq 0.05$), RBC, HGB, HCT, and PLT increased very significantly ($P \leq 0.001$) in the 300 mg/kg-treated group. However there were still no significant changes in MCV, MCH, MCHC, and RDW with the treatments (Table 4).

Table 2: The haematological profiles of New Zealand White rabbits prior to phlebotomization (Normal I), phlebotomized rabbits (PBTM), and normal rabbits kept under experimental conditions over the 40-day study period (Normal II).

Variables	Normal I	PBTM	Normal II
WBC (K/μL)	8.7±1.5	5.3±1.7††	8.0±1.8^{ns}
RBC (M/μL)	6.6±0.8	4.0±0.8†††	6.3±0.9^{ns}
HGB (g/dL)	13.2±0.7	8.3±0.8†††	12.9±1.0^{ns}
HCT (%)	42.3±3.5	27.3±3.9†††	40.8±3.6^{ns}
MCV (fL)	64.2±2.3	63.4±3.5^{ns}	64.9±3.2^{ns}
MCH (pg)	21.5±2.6	21.9±3.0^{ns}	22.5±3.6^{ns}
MCHC (g/dL)	33.9±2.3	35.2±2.9^{ns}	34.5±3.5^{ns}
RDW (%)	16.2±1.9	16.6±2.1^{ns}	16.4±1.8^{ns}
PLT (K/μL)	379±55.8	232±43.6†††	342±48.8^{ns}

Values quoted are means ± SD. Levels of significance between phlebotomized/normal II and normal I was determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant decrements: †† implies P ≤ 0.01; ††† implies P≤ 0.001.

DISCUSSION

The significant reduction in the blood cells in circulation after phlebotomy is an indication that this protocol causes blood cell deficiency which will require haematopoiesis for correction. The very significant elevation of WBC, RBC, and PLT caused by the 300 and 1000 mg/kg doses of EIE and the reference hematinic indicates enhanced haematopoiesis. This indicates that EIE can be used to correct anaemia resulting from active bleeding, malnutrition, pregnancy, and chronic disease; leucopenia caused by viral infection, rheumatoid arthritis and autoimmune disorders; and thrombocytopenia caused by diminished platelet survival. Haematopoiesis requires iron and the B-complex vitamins (Koury and Ponka, 2004), folic acid, some mineral such as copper, zinc and manganese, and the action of haematopoietic growth factors or haematopoietic cytokines. Similarity in effect between the higher doses of the extract and the reference hematinic suggests that the extract could have some of the components stated or could possibly stimulate the synthesis and release of the haematopoietic growth factors (which are synthesized by activated cells under required conditions rather than being produced constitutively all the time) to cause the proliferation and differentiation of haematopoietic stem cells of the bone marrow into the various blood cells.

Although the body's homeostatic mechanisms could induce haematopoiesis (seen as the slight elevation of blood cells in the normal saline treated rabbits), the extracts and the reference hematinic induced haematopoiesis very significantly in a

Table 3: A comparison of the haematological profiles of phlebotomized (PBTM) rabbits and phlebotomized rabbits treated with 0.23 ml/kg normal saline (NS), 0.23 ml/kg Feroglobin®, and 100, 300, and 1000 mg/kg EIE for 20 days.

Variables	PBTM	NS	Feroglobin®	EIE (100)	EIE (300)	EIE (1000)
WBC(K/µl)	5.3±1.7	6.3±1.4^{ns}	9.0±1.3††	7.2±1.2^{ns}	8.0±1.3†	8.0±1.5†
RBC(M/µl)	4.0±0.8	4.4±0.6^{ns}	5.8±0.6††	4.8±0.7^{ns}	5.4±0.7†	5.6±0.9††
HGB (g/dl)	8.3±0.8	9.0±0.8^{ns}	11.6±0.8†††	9.8±0.7†	10.9±0.7†††	11.3±0.9†††
HCT (%)	27.3±3.9	30.7±2.9^{ns}	39.6±1.9†††	32.1±2.4†	37.0±2.4†††	37.1±1.8†††
MCV (fl)	65.4±3.5	66.8±2.9^{ns}	65.5±3.2^{ns}	65.2±3.5^{ns}	66.8±2.6^{ns}	65.4±3.8^{ns}
MCH (pg)	20.0±1.0	19.5±1.4^{ns}	19.9±1.9^{ns}	19.3±1.1^{ns}	19.8±1.2^{ns}	19.9±1.0^{ns}
MCHC (g/dl)	31.2±2.9	31.6±2.1^{ns}	31.2±2.6^{ns}	31.3±2.4^{ns}	31.7±2.9^{ns}	31.4±2.7^{ns}
RDW (%)	16.6±2.1	16.4±1.7^{ns}	16.4±2.2^{ns}	16.8±1.5^{ns}	16.0±1.9^{ns}	16.6±2.1^{ns}
PLT (K/µl)	232±43.6	278±51.8^{ns}	365±62.7††	291±47.8^{ns}	380±56.2††	388±72.5††

Values quoted are means ± SD. Levels of significant between the phlebotomized and the phlebotomized but treated rabbits were determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant increments: † implies P ≤ 0.05; †† implies P ≤ 0.01; ††† implies P ≤ 0.001; ns implies P > 0.05.

Table 4: A comparison of the haematological profiles of phlebotomized (PBTM) rabbits treated with 0.23 ml/kg normal saline (NS), 0.23 ml/kg Feroglobin®, 100, 300, and 1000 mg/kg EIE for 40 days.

Variables	PBTM	NS	Feroglobin®	EIE (100)	EIE (300)	EIE (1000)
WBC(K/µl)	5.3±1.7	6.9±1.3^{ns}	8.8±1.3††	7.8±1.5†	8.1±1.4†	8.5±1.2††
RBC(M/µl)	4.0±0.8	5.5±0.9†	7.3±0.9†††	5.9±0.9††	6.5±0.7†††	6.8±0.9†††
HGB (g/dl)	8.3±0.8	10.8±1.3†	14.5±1.2†††	11.9±1.7††	13.3±1.1†††	14.1±1.9†††
HCT (%)	27.3±3.9	36.1±3.4††	48.7±3.9†††	40.4±2.8†††	43.7±2.7†††	45.9±3.7†††
MCV (fl)	65.4±2.0	66.2±1.6^{ns}	66.4±1.5^{ns}	66.1±1.7^{ns}	66.7±1.4^{ns}	65.8±1.7^{ns}
MCH (pg)	20.0±1.0	19.3±1.6^{ns}	19.5±1.5^{ns}	19.9±1.2^{ns}	20.1±1.8^{ns}	19.2±1.1^{ns}
MCHC(g/dl)	31.2±2.9	29.8±1.1^{ns}	30.4±1.3^{ns}	30.7±1.4^{ns}	29.9±1.1^{ns}	30.1±1.2^{ns}
RDW (%)	16.6±2.1	16.9±1.9^{ns}	16.1±1.4^{ns}	16.6±2.1^{ns}	16.5±1.7^{ns}	16.2±1.8^{ns}
PLT (K/µl)	232±43.6	303±49.7^{ns}	419±52.2†††	324±48.4†	362±58.6††	388±64.5†††

Values quoted are means ± SD. Levels of significant between the phlebotomized and the phlebotomized but treated rabbits were determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant increments: † implies P ≤ 0.05; †† implies P ≤ 0.01; ††† implies P ≤ 0.001.

shorter duration of time. The insignificant changes in haematological profile between the normal animals prior to phlebotomy and normal rabbits kept under experimental conditions indicates that the experimental conditions had no adverse effect on the haematological profile of experimental animals.

Preliminary phytochemical screening carried out in this study indicated that *Ipomoea involucrata* leaves contain tannins, alkaloids, glycosides, and saponins in its ethanolic extract. The haematopoietic potential of the leaf extract may be related to its phyto chemicals present. Glycosides and saponins have been documented to significantly increase the proliferation abilities of bone marrow cells (Kirby and Bentley, 1991; Gao *et al.,* 1992; Li *et al.,* 2011). Alkaloids enhance the restoration of haematopoiesis (Boyko and Belskiy, 1998). Tannins have been reported to inhibit the formation of superoxide ions and hydroxy radicals, which are two strong peroxidation agents (Facino *et al*., 1990; Uboh *et al.,* 2010). This antioxidant activity may protect both the haematopoietic committed stem and the formed blood cells from the attack of the reactive free radicals in the body.

CONCLUSION

The ethanolic leaf extract of *Ipomoea involucrata* has some haematopoietic effects and thus can be used to treat anaemia and other blood cell deficiency disorders. Its safety for use however needs to be ascertained.

ACKNOWLEDGEMENT

I wish to acknowledge the support I received in the pharmacology laboratory from the technicians Mr. Thomas Ansah and Mr. Prosper Akwatia.

REFERENCES

Bodekar GC, (1994). Traditional health care and public policy: recent trend. A paper presented at International Conference Canada and edited by Anwar I. in Traditional Health System and Public Policy, international Development Research Center, Ottawa, Canada.

Bouquet A, (1969). Feticheurs et Medicines Tradition alles du Congo (Brazzaville). O.R.S.T.O.M., Memoire: 99–100.

Bouquet A, Debray M, (1974). Plantes Medicinalis de Cote d´Ivoire. *Document Orstrom 32: 76*.

Boyko VN, Belskiy SN, (1998). The influence of the novel drug Ukrain on hemo- and immunopoiesis at the time of its maximum radioprotective effect. Drugs Exp Clin Res., 24(5-6): 335-337.

Burkill HM, (1985). The useful plants of west tropical Africa. *Vol 1 Royal Botanic Gardens, Kew (K)*

Commey JO, Dekyem P (1995). Childhood deaths from anaemia in Accra, Ghana. *West African J Med., 14:101-4*.

de Benoist B, McLean E, Egli I, Cogswell M (2008).Worldwide prevalence of anaemia 1993-2005 WHO Global Database on Anaemia.

Dokosi OB, (1998). Herbs of Ghana. Ghana University Press, Accra, Ghana.

Facino RM, Carini M, Franzoi L, Pirola O, Bosisio E, (1990). Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum* G. Don (Compositae). *Pharmacol Res.*, 22(6):709-721.

Galloway R (2003). Anaemia prevention and control - What works? Part 1 | Part 2 USAID, World Bank, PAHO/WHO, Micronutrient Initiative, FAO, and UNICEF.

Gao RL, Xu CL, Jin JM, (1992). Effect of total saponins of Panax ginseng on haematopoietic progenitor cells in normal human and aplastic anaemia patients. *Zhongguo Zhong Xi Yi Jie He Za Zhi.* 12(5): 285-7, 261-2.

Harborne JB, (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd Edition, Springer, London, p 302.

Kirby SL, Bentley SA, (1991). Xyloside effects on in vitro haematopoiesis: functional and biochemical studies. *J Cell Physiol.,* 148(1):116-123.

Koury MJ, Ponka P (2004). New Insights into Erythropoiesis: The Roles of Folate, Vitamin B_{12}, and Iron. *Annual Review of Nutrition; 24: 105-131*

Li YR, Cao W, Guo J, Miao S, Ding GR, Li KC, Wang J, Guo GZ, (2011). Comparative investigations on the protective effects of rhodioside, ciwujianoside-B and astragaloside IV on radiation injuries of the haematopoietic system in mice. *Phytother Res.* 25(5): 644-653.

McGuill MW, Rowan AN, (1989). Biological effects of blood loss: Implication for sampling volumes and techniques. *ILAR News., 31(4): 5-20*.

Morimoto M (2009). General Physiology of Rabbits, In: Louis-Marie Houdebine, Jianglin Fan. Rabbit Biotechnology: Rabbit Genomics, Transgenesis, Cloning and models, Springer Science, London, New York, p 32.

Obeng SK, 2008. Rural banking in Ghana: it's impact on rural farmers (A case study of Abokobi rural area). Samven's Research and Consultancy Unit. p 4

Oliver B (1960). Medicinal Plants in Nigeria, College of Arts, Science and Technology, Lagos, p *68*.

Stoltzfus RJ, (2003). Iron deficiency: global prevalence and consequences. *Food Nutr Bull,* 24 (4suppl):S99–103

Suckow MA, Brammer DW, Rush HG, Chrisp CE. Biology and diseases of rabbits, In: Fox JG, LC Anderson, FM Loew, FW Quimby, 2002. Laboratory animal Medicine, 2nd edition, Academic Press, San Diego (CA). p 334.

Uboh FE, Okon IE, Ekong MB, (2010). Effect of Aqueous Extract of *Psidium Guajava* Leaves on Liver Enzymes, Histological Integrity and Haematological Indices in Rats. *Gastroenterology Research* (3)1: 32-38.

World Health Organization. *Traditional Medicine.* WHO Fact Sheet No. 134. 2008 Geneva Available at: http://www.who.int/mediacentre/factsheets/fs134/en/ Accessed on December 21, 2011.

Zhang X, (1994). WHO Policy and Activities on Traditional Medicines, An article on traditional health system and public policy, International Development Research Center, Ottawa, Canada.

ISSN 2026-6294

Journal of Medical and Biomedical Sciences (2018) 7(2): 17-28

ORIGINAL ARTICLE

Residual Risk of Transmission of Hepatitis B Virus through Blood Transfusion in Ghana: Evaluation of the performance of Rapid Immunochromatographic Assay with Enzyme Linked Immunosorbent Assay

W.K.B.A. Owiredu[1], J. Osei-Yeboah[2], N. Amidu[3] and E.F. Laing[1]

[1]Department of Molecular Medicine, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; [2]Department of Medical Laboratory Technology, College of Health, Kintampo, Ghana; [3]Department of Medical Laboratory Sciences, School of Medicine and Health Sciences, University for Development Studies, Tamale, Ghana

Blood transfusion necessitates screening of transmissible infectious pathogens such as hepatitis B virus (HBV) to curtail post transfusion risk of infection. The study re-examined this approach by evaluating the efficiency of solely testing for hepatitis B surface antigen (HbsAg) marker for blood transfusion, the efficacy of the various immunochromatographic assays in the screening process and the residual risk of hepatitis B viral transmission through transfusion in Ghana. A convenient purposive sampling technique was used in selecting ten hospitals, from each of the 10 regions. A total of 480 aliquots of blood were collected anonymously, from blood already tested for HbsAg with immunochromatographic assay in the blood banks of the chosen facilities and declared negative. Plasma from the blood was obtained through centrifugation, separated into well labeled microtubes and transported in cold boxes to the Molecular Medicine Department-KNUST. The samples were then re-examined for all six hepatitis B virus (HBV) (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc and IgM anti-HBc) serological markers using ELISA assay. When a total of 480 plasma samples from the blood banks of the ten chosen facilities were re-examined with the ELISA assay, 39(8.13%) samples reacted positive for HBsAg, 60(12.5%) reacted for Anti-HBs, 13(2.71%) reacted for HBeAg, 51(10.63%) for Anti-HBe and 329(68.54%) reacted positively for Anti-HBc. None of the samples reacted positive for IgM anti-HBc. The estimated sero-prevalence for all HBV serological markers is 76.67% whereas the estimated residual risk of HBV infection through blood transfusion caused by the use of immunochromatographic methods in the screening of blood for transfusion was 8.47%(5.98% - 10.94% at 95% CI). An additional risk of 3.10%(1.54% - 4.62% at 95% CI) of HBV infection through transfusion was also estimated for the non-testing of other HBV infectious serological markers. The total residual risk for transfusion transmitted HBV was 11.16%(8.34% - 13.95% at 95% CI). The study revealed that neither the kits in use nor the testing strategy in place now is adequate to prevent transmission of hepatitis B virus through transfusion in Ghana due to the high residual risk of transmission of HBV. There is therefore an urgent need for a sustainable quality control system on the screening of HBsAg in blood for donation in Ghana.
Journal of Medical and Biomedical Sciences (2018) 7(2), 17-28

Keywords*:* Blood transfusion, HBsAg, immunochromatographic , ELISA, Blood bank, Ghana

Correspondence: *W.K.B.A. Owiredu, Department of Molecular Medicine, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. E*-mail: wkbaowiredu@yahoo.com

INTRODUCTION

Transfusion transmitted infections are still a major global public health concern confronting the worldwide delivery of transfusion services and this is

even more apparent in the under resourced transfusion centres operating in the third world countries (Aach *et al.*, 1981). Blood transmitted infections involving pathogenic viruses are the most renowned in transfusion medicine (Niederhauser *et al.*, 2005). In spite of all the scientific advancement geared towards blood donation safety, Hepatitis B virus residual risk remains the single most transmitted infectious pathogenic disease passed on from blood donors to their recipients through transfusion (El-Sherif *et al.*, 2007).

Hepatitis B virus infection leads to a sequence of serological markers or viral protein in the bloodstream of infected individuals. Hepatitis B viral deoxyribonucleic acid (DNA) is the first to appear in the blood, then hepatitis B viral surface antigen (HBsAg), followed by viral polymerase DNA and then hepatitis B viral e antigen (HBeAg). Host's response results in production of antibodies to various hepatitis B viral antigens. The first detectable antibody in the serum of an infected person is antibody to the core antigen (Anti-HBc); this antigen is not expressed in the bloodstream and is only found in HBV-infected hepatocytes in the liver. Antibodies to hepatitis B viral e antigen (Anti-HBe) are then secreted into the serum and followed finally by antibody to hepatitis B viral surface antigen (Anti-HBs) (Kumar *et al.*, 2007; Kukka, 2008).

The issues with blood safety are essentially in two parts. First and most important is the identification of tainted or infectious blood units and the onward prevention of its transfusion. In the presence of safeguarding the recipient, steps must also be taken to prevent false positives which could lead to wastage of blood. The donor selection policy adopted by the facility should therefore be able to exclude donors from high risk groups. Also the specificity and sensitivity of the assay technology in use should be high enough to identify all true positive and true negative blood donors or blood units (Allain and Lee, 2005).

Despite the institution of mandatory screening for HBsAg, the issue of transfusion-associated HBV is still a major health problem plaguing most third world and resource poor countries (Dhawan *et al.*, 2008). Blood donors in Ghana are selected on the basis of a health check questionnaire coupled with a visual physical examination and a mandatory microbiological screening which includes Syphilis, HIV, Hepatitis C and HBsAg test (Ampofo *et al.*, 2002).

Among the pathogenic markers which are screened before transfusion in Ghana, human immunodeficiency virus screening is the only marker that has a nationally developed standardised guideline procedure for testing, supervision and centralised provision of test kits (Allain *et al.*, 2003). Unlike HIV, Hepatitis B virus surface antigen (HBsAg) screening in Ghana does not have any nationally developed supervised procedures and thus the choice of assay technology and brand of test kit used is the sole prerogative of health facilities (Ampofo *et al.*, 2002).

Retrospective studies carried out in other countries on blood donors using first generation tests such as immunodiffusion and/or immunochromatography in the detection of HBsAg, found that 52%-69% of recipients of blood developed hepatitis B infection (Alter and Chalmers, 1981). Allain and co-workers in 2003 observed that neither the latex agglutination nor the dipstick assays currently in use for HBsAg screening had sufficient sensitivity in excluding HBV infected blood from transfusion. Latex agglutination method presented a false negative rate of 46%, whilst dipstick recorded a rate of 29% false negative (Allain et al., 2003). HBsAg sero-negative test however does not rule out the risk of transmission of hepatitis B infection (Al-Mekhaizeem *et al.*, 2001; Brechot *et al.*, 2001; Kumar *et al.*, 2007), and this can be as a result of undetected infected donors with early acute infection in the "window period", resolving infection, silent or occult infection or infection with atypical variants or mutation (Reesink *et al.*, 2008).

The accurate estimation of residual risk of transfusion-transmissible infectious diseases are essential for monitoring the safety of blood supply and the evaluation of the potential effect of screening tests and the risk reduction procedures under implementation (Schreiber *et al.*, 1996).The most direct way of estimating the residual risk of viral agents transmissible

through transfusion would be a prospective study that evaluates the rate of viral infection in blood recipients (Aach *et al.*, 1981; Donegan *et al.*, 1990). However such a prospective study would require testing a large patient population for HBV before transfusion and then follow up testing after transfusion for the risk to be measured accurately and this would be both resource and time demanding. There is also an obvious implication of responsibility for health facilities where recipient after transfusion later test positive for HBV and these make such research impracticable in most settings. The alternative therefore is further retesting for the rate of infection in samples of donated blood that test negative on routine screening by the use of more sensitive assays for the viral agents (Schreiber *et al.*, 1996). The primary objective of this study therefore was to evaluate the residual risk of transmission of hepatitis B virus through blood transfusion and thus assess the efficacy of the test methods being used for HBsAg screening and the efficiency of using HBsAg as sole hepatitis B viral marker for blood transfusion in Ghana.

MATERIALS AND METHODS

Sampling

A convenient, purposive cross-sectional study was carried out from October 2007 to June 2008 in ten laboratories, one in each of the ten regions in Ghana. The study was anonymous and non-linked. Aliquots of blood were collected from donated blood units that tested sero-negative for HBsAg upon screening with immunochromatography assays in the facilities. The plasma was separated into well labelled micro-tubes and transported in cold boxes to the Molecular Medicine Laboratory of the Kwame Nkrumah University of Science and Technology in Kumasi and stored at -20 degrees. The types and brand names of the immunochromatogrphic assays used for the initial screening at the various facilities were recorded. Samples were re-examined for HBsAg and the five other serological markers of HBV (Anti-HBs, HBeAg, Anti-HBe, Anti-HBc and IgM Anti-HBc) using an Intec advanced 3rd generation ELISA test kit according to the manufacturers instruction. All blood donors in Ghana are of consenting age and consented to the retesting of HBV. Approval for this study was granted by the research and coordinating units of all ten laboratory facilities involved in the conduct of the study.

Dipstick assay methods

All samples from the Central, Ashanti and Brong Ahafo regions were pre-screened with DiaSpot™ one step test strip (DiaSpot World of Health Biotech Co.; USA). Samples from the Volta and Upper East regions were screened with Virucheck™ dipstick, (Orchid Biomedical Systems, India). Samples from the Greater Accra and the Eastern regions were screened with Clinotech™ HBsAg Dipstick, (Clinotech Diagnostics, Canada). Samples from the Northern regions were screened with one step HBsAg dipstick (Wondfo™ Guangzhou Wondfo Biotech Co., Ltd. China). Samples from the Upper West were screened with Novatec™ dipstick (Novatec Immundiagnostica GmbH, Technologie & Waldpark. Germany) and samples from the Western region were screened with Accull-Tell™ test kits. (AccuBiotech Co. Ltd. China). The strips were dipped in the serum or plasma for about 10 seconds and read for the visibility of one test and one procedural control line after about 10 minutes.

Enzyme Linked Immunosorbent Assay (ELISA)

HBsAg, Anti-HBs and HBeAg were determined by a double sandwich enzyme-linked immunosorbent assay (ELISA) using Advanced™ 3rdgeneration assay kits (InTec Products Inc. China). Anti-HBe and Anti-HBc were determined by competitive immunoassay using Advanced™ 3rd generation assay kits (InTec Products Inc.China). Anti-HBc IgM was tested with an immunoassay using Advanced™ 3rd generation assay kits (InTec Products Inc.China). Reactive samples were retested in duplicate and considered to be reactive if at least 1 of the 2 repetitions also gave a positive result.

Statistical analysis

Statistical analysis was performed using MedCalc Version 10.2.0.0 for Windows 98/NT/Me/2000/XP/Vista (Vienna, Ausra. http://www.medcalc.be). The Bland-Altman analysis was used to compare

the immunochromatographic method for the screening of HBsAg and ELISA screening for HBsAg for each region. The calculation of the residual risk of HBV transmission through blood transfusion was done using the Modified Wald analysis for categorical outcomes as recommended by Agresti and Coull, (1998).

RESULTS

Reactivity of Hepatitis B viral marker in the various regions upon re-examination

As shown in Table 1, out of the total number of 480 samples that had tested sero-negative upon testing with rapid immunochromatographic test kit in the various facilities and thus the blood declared safe for transfusion, 39(8.13%) of the samples reacted sero-positive for HBsAg upon retesting with ELISA. Sixty (12.5%) of the sample showed sero-positivity for Anti-HBs, 13(2.71%) reacted for HBeAg, 51 (10.63%) for Anti-HBe, 329(68.54%) reacting sero-positively for Anti-HBc with 226(47.08%) reacting sero-positive for isolated or only Anti-HBc. None of the samples however reacted sero-positive to IgM anti-HBc. The Volta region 10(20.00%), the Northern region 6(13.95%), the Upper East region 7(13.46%) and the Eastern region 5(10.00%) all showed a higher HBsAg seroreactivity than the total percentage seroreactivity of 39(8.13%) from all centres.

Comparison of dipstick methods with ELISA method using Bland-Altman analysis

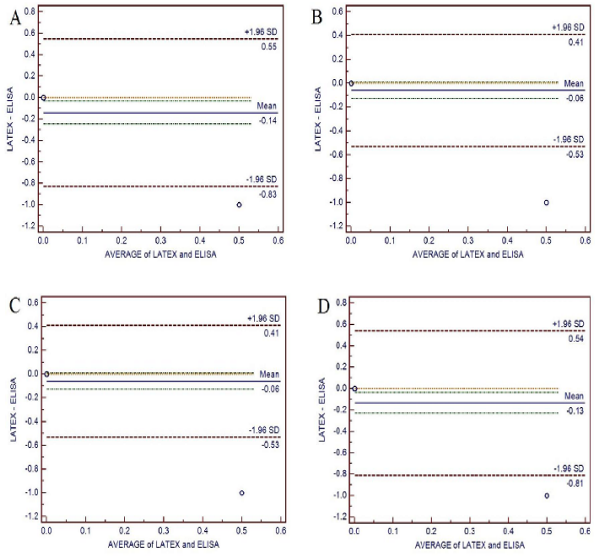
Table 1: Reactivity of Hepatitis B viral marker in the various regions upon re-examination

Region	Sample	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	Isolated Anti-HBc
Eastern	50	5(10.00)	10(20.00)	1(2.00)	0(0.00)	40(80.00)	25(50.00)
Volta	50	10(20.00)	1(2.00)	3(6.00)	1(2.00)	19(38.00)	13(26.00)
Central	50	0(0.00)	3(6.00)	0(0.00)	1(2.00)	22(44.00)	19(38.00)
Northern	43	6(13.95)	0(0.00)	3(6.98)	6(13.95)	37(86.05)	29(67.44)
Greater Accra	50	0(0.00)	5(10.00)	1(2.00)	11(22.00)	35(70.00)	22(44.00)
Ashanti	50	3(6.00)	12(24.00)	2(4.00)	8(16.00)	28(56.00)	15(30.00)
Western	42	3(7.14)	6(14.29)	0(0.00)	6(14.29)	31(73.81)	22(52.38)
Upper West	43	2(4.65)	9(20.93)	1(2.33)	5(11.63)	32(74.42)	25(58.14)
Brong Ahafo	50	3(6.00)	8(16.00)	2(4.00)	8(16.00)	40(80.00)	26(52.00)
Upper East	52	7(13.46)	6(11.53)	0(0.00)	5(9.62)	45(86.54)	30(57.69
Total	480	39(8.13)	60(12.50)	13(2.71)	51(10.63)	329(68.54)	226(47.08

Data are presented as number or number (percentage). HBsAg = Hepatitis B virus Surface antigen, Anti-HBs = Antibody to Hepatitis B virus surface antigen, HBeAg = Hepatitis B virus e antigen, Anti-HBe = Antibody to Hepatitis B virus e antigen, Anti-HBc = Antibody to hepatitis B virus core antigen

When the results for HBsAg screening using the rapid latex immunochromatographic method was compared with the ELISA assay using the Bland-Altman analysis and as shown in figures 1 and 2. The Volta region (ViruCheck) showed the highest underestimation with a bias of -0.20 with a 95% limit of agreement of -0.99 to 0.59 (figure 2D). Northern Region (Wondfo), also underestimated presence of HBsAg in the samples (-0.14 and the 95% limit of agreement was -0.83 to 0.55, Figure 1A). Upper East region (Virucheck) generated underestimated HBsAg sero-reactivity with a bias of -0.13 and 95% limit of agreement of -0.81 to 0.54 (Figure 1D). This was followed by Eastern region (Clinotech) generating a bias of -0.10 and the 95% limit of agreement was -0.69 to 0.49 (Figure 2B). Western region (Acull-Tell) showed a bias of -0.07 and the 95% limit of agreement was -0.58 to 0.44 (Figure 2C). The bias was -0.06 and the 95% limit of agreement were -0.53 to 0.41 for both Ashanti and Brong Ahafo regions (DiaSpot) (Figure 1B and 1C respectively). A bias of -0.05 and a 95% limit of

agreement of -0.46 to 0.37 was generated in the Upper West region (Novatec) (Figure 2A). There was perfect agreement between the immunochromatographic methods used in both Central and Greater Accra region facilities and the ELISA method.



A = Northern region, B = Ashanti region, C = Brong Ahafo region and D = Upper east region

Figure 1: Bland-Altman graphs of difference scores for the screening of the Hepatitis B viral antigen by the various Rapid immunochromatographic assays evaluated with the ELISA Assay

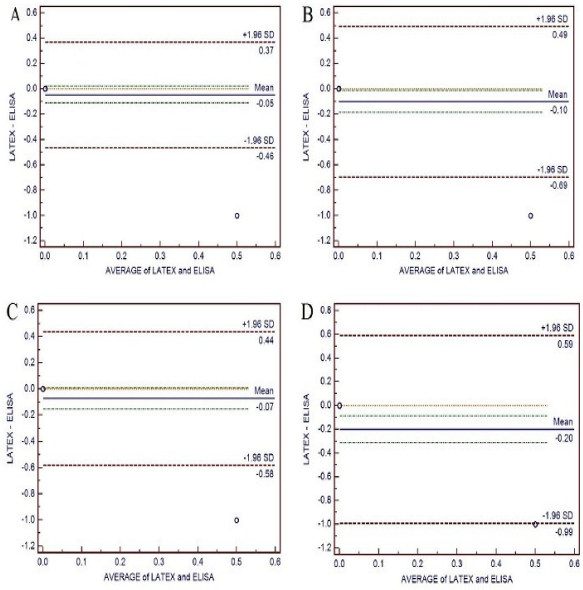


Figure 3: Bland-Altman graphs of difference scores for the screening of the Hepatitis B viral antigen by the various Rapid immunochromatographic assays evaluated with the ELISA Assay. A = Upper West region, B = Eastern region, C = Western region, D = Volta region

Residual risk of transfusion

The modified Wald analysis was used to estimate the residual risk of transmission of HBV through blood transfusion for the use of rapid immunochromatographic test kits in screening for the HBsAg before transfusion. As shown in Table 2, the national mean residual risk of transmission of HBV was 8.47% (5.98% to 10.94% at 95% CI).The facility at the Volta region (VR) generated the highest residual risk of transmission, 22.22% (11.05% to 33.23% at 95% CI) and followed in a descending order of magnitude, the residual risk of HBV transmission through blood transfusion in the facility at the Northern region (NR), was 17.02 % (6.18% to 27.64% at 95% CI), the Upper East region (UE), 16.07% (6.37% to 25.58% at 95% CI), the Eastern region (ER), 12.96 % (3.91% to 21.79% at 95% CI), the Western region (WR), 10.87% (1.77% to 19.70% at 95% CI). The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region generated a residual risk of 9.26% (1.44% to 16.84% at 95% CI). The facility in the Upper West region (UW) generated a residual risk of 8.51% (0.44% to 16.30% at 95% CI). With the exceptions of the two facilities in the Central and the Greater Accra regions which generated a residual risk of 3.70% (0.00% to 8.52% at 95% CI) each, all the other facilities had a residual risk higher than the national mean residual risk of HBV transmission through transfusion.

To assess the risk of transmission of HBV for solely testing for hepatitis B surface antigen (HBsAg) marker for blood transfusion without testing the other HBV serological markers (anti-HBs, HBeAg,

anti-HBe, anti-HBc and IgM anti-HBc), two serological markers which are indicators of the presence of the virus and thus a risk of transfusion hepatitis infections were considered in the Modified Wald analysis. These were reactivity of HBeAg which is serologically indicative of high HBV replication and IgM anti-HBc which indicates an acute infection of HBV. Thus samples that showed sero-reactivity for HBeAg and IgM anti-HBc without HBsAg were considered.

The total percentage residual risk of HBV transmission for using the HBsAg as the sole HBV marker to transfused blood was 3.10 %(1.54% to 4.62% at 95% CI). The facility at the Northern region (NR) generated the highest residual risk of transmission, 10.64% (1.72% to 19.29% at 95% CI) and followed in a decreasing order of magnitude, the residual risk of HBV transmission through blood transfusion in the facility at the Volta region (VR),was 9.26% (1.44% to 16.84% at 95% CI). The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region (BA) generated a residual risk of 7.41% (0.34% to 14.22% at 95% CI) for non inclusion of the two markers in the testing of HBV blood transfusion. Again the facilities in the Greater Accra region (GA) and the one in the Eastern region (ER) each generated a residual risk of 5.56% (0.01% to 11.47% at 95% CI). The residual risk for the facility in the Western region (WR) was 4.35% (0.00% to 9.99% at 95% CI) and that for the Central region (CR) was 3.70% (0.00% to 8.52% at 95% CI). A residual risk of 3.57% (0.01% to 13.16% at 95% CI) and 3.57% (0.00% to 8.22% at 95% CI) were obtained for the two facilities in the Upper West region (UW) and the Upper East region (UE) respectively. As shown in table 2. All the individual facilities generated a residual risk higher than the total average.

In the estimation of the total residual risk of HBV transmission through transfusion, the samples that reacted sero-positive for any of the infectious HBV serological markers (HBsAg, HBeAg and IgM anti-HBc) were considered and counted only once. Though the risk of transmission is not nil for isolated anti-HBe sero-positive samples in the absence of HBsAg positivity, however the inability to establish the presence or otherwise of viral DNA in these samples by the test method (ELISA) led to the exclusion of such samples. Also sero-reactivity for anti-HBc was excluded due to the inability of the test method (ELISA) to distinguish between samples that tested sero-positive for anti-HBc and sero -negative for all other HBV markers indicative of infectivity (HBsAg, HBeag, and IgM anti-HBc) but contained viral DNA and thus infectious, from those that exhibited the same serological profile but lack viral DNA.

As shown in Table 2, the total percentage residual risk was estimated as 11.16% (8.34% to 13.95% at 95% CI). The total residual risk of transmission of the individual facilities were 27.78% (15.76% to 39.67% at 95% CI) for VR, 23.40% (11.21% to 35.42% at 95% CI) for NR, 16.07% (6.37 to 25.58% at 95% CI) for UE. A total residual risk of 16.07% (1.72% to 19.29% at 95% CI) and 14.82% (5.25% to 24.17% at 95% CI) for the Upper West (UW) and the Eastern regions (ER) respectively. The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region (BA), each generated a total residual risk of 12.96% (3.91% to 21.79% at 95% CI).The facilities Western, Greater Accra and Central regions all had their total residual risk below the national average, 10.87% (1.77% to 19.70% at 95% CI) for WR, 5.56% (0.01% to 11.47% at 95% CI) for GA and 3.70% (0.00% to 8.52% at 95% CI) for CR.

Reactivity Profile of ELISA upon Retesting

The reactivity profile of the samples upon retesting with enzyme linked immunosorbent assay showed disparity between the number of samples that reacted for a particular antigen and its corresponding antibody. Figure 3A and Figure 3B represents regional reactivity profile of HBV surface antigen and its corresponding regional reactivity profile of surface antibody respectively, whilst Figure 3C and Figure 3D represents regional reactivity for HBV e antigen and e antibody respectively. There were a higher number of samples that reacted for anti-HBc only (Figure 3F) compared to the sample that reacted for anti-HBc and other serological markers (Figure 3E). There was high discordance between HBsAg and HBeAg with only three of the samples

Table 2: Estimated Residual Risk of Hepatitis B virus transmission through blood transfusion

LOCATION	RRT for testing HBsAg with Immunochromato-graphic Assays	RRT for the non testing of other HBV serological markers	Total RRT of HBV through Transfusion
Eastern	12.96(3.91 - 21.79)	5.56(0.01 - 11.47)	14.82(5.25 - 24.17)
Volta	22.22(11.05 - 33.23)	9.26(1.44 - 16.84)	27.78(15.76 - 39.67)
Central	3.70(0.00 - 8.52)	3.70(0.00 - 8.52)	3.70(0.00 - 8.52)
Northern	17.02(6.18 - 27.64)	10.64(1.72 -19.29)	23.40(11.21 - 35.42)
Greater Accra	3.70(0.00 - 8.52)	5.56(0.01 - 11.47)	5.56(0.01 - 11.47)
Ashanti	9.26(1.44 - 16.84)	7.41(0.34 - 14.22)	12.96(3.91 - 21.79)
Western	10.87(1.77 - 19.70)	4.35(0.00 - 9.99)	10.87(1.77 - 19.70)
Upper West	8.51(0.44 - 16.30)	3.57(0.01 - 13.16)	16.07(1.72 - 19.29)
Brong Ahafo	9.26(1.44 - 16.84)	7.41(0.34 - 14.22)	12.96(3.91 - 21.79)
Upper East	16.07(6.37 -25.58)	3.57(0.00 - 8.22)	16.07(6.37 - 25.58)
Total	8.47(5.98 - 10.94)	3.10(1.54 - 4.62)	11.16(8.34 - 13.95)

Data are presented in percentage, RRT Residual risk of transmission

from the northern region showing concordance for HBsAg (Figure 3G). There was high reactivity correlation between Anti-HBs to the Anti-HBc (Figure 4D). Anti-HBe also showed higher reactivity correlation to Anti-HBc (Figure 4G) than Anti-HBs.

DISCUSSION

The year 2012 has been targeted as the year for safe blood and the achievement of 100% testing for infectious markers by the World Health Organization (WHO) (Tagny *et al*., 2008). Though these may look achievable in the developed world, sub-

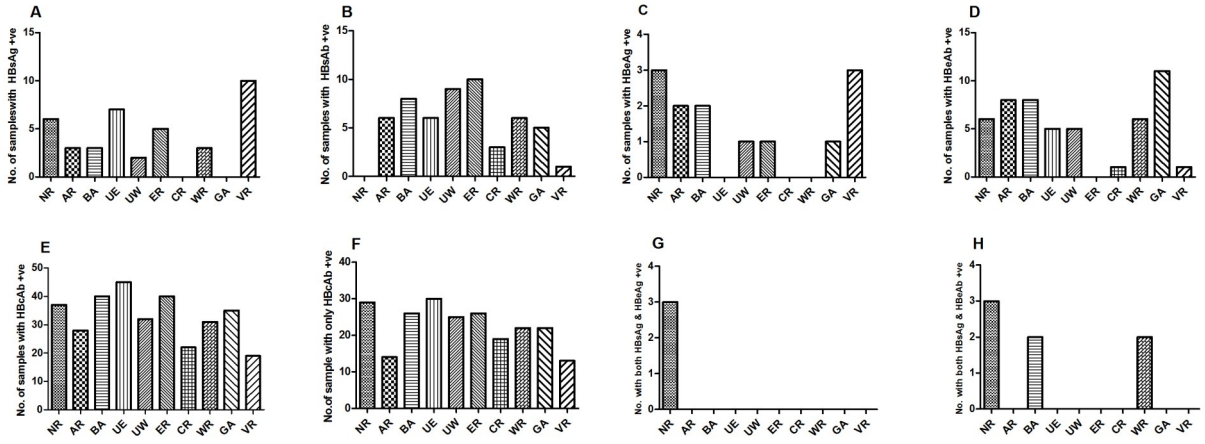


Figure 3: Hepatitis B Viral Serological Marker Reactivity Profile of Enzyme Linked Immuno-Sorbent Assay upon Retesting

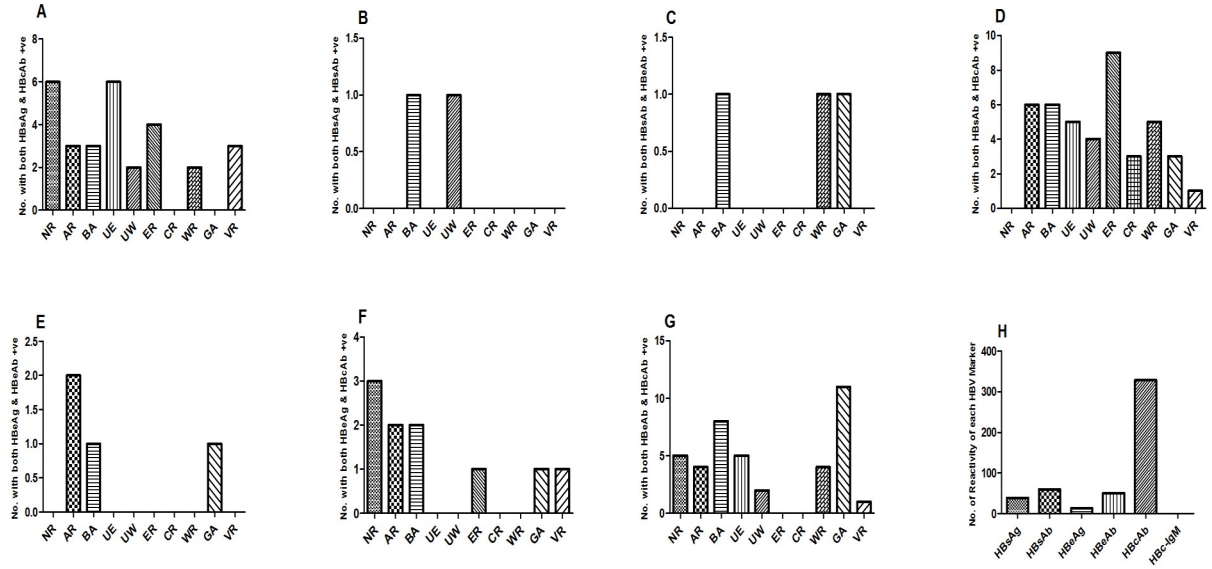


Figure 4: Hepatitis B Viral Serological Marker Reactivity Profile of Enzyme Linked Immuno-Sorbent Assay upon Retesting

Saharan Africa remains drawn back, since it is confronted with multi-factorial issues that compromise blood safety in most of the countries. The most important of these borders on the deficient screening of donor blood for infectious markers like hepatitis B and C virus, in an environment already plagued by a high burden of blood-borne infections and poverty (Tagny *et al.*, 2008).

Even though by the study design the immunochromatographic assay sorted out many of the HBsAg positive samples and its accompanied other HBV serological markers, an estimated sero-prevalence of 76.67% for all HBV serological markers was observed; this is in agreement with previous work by Martinson *et al.,*(1998) who reported a sero-prevalence of 74.7% for all HBV serological markers for a rural community in the Ashanti region. Upon retesting with the ELISA, 8.13% of the samples reacted sero-positive for HBsAg. Amidu *et al.*, (2010) in a four year retrospective study from 2004 to 2007 reported a hepatitis B virus sero-prevalence of 12.64% among blood donors in the upper east region using rapid immunochromatographic assay. Thus it can be said that prevalence of hepatitis B virus among blood donors was under estimated by 8.13% in general and 13.46% in particular to Upper East region.

False negative HBsAg results were obtained by six different brands of rapid latex immunochromatographic assays that were used in all the ten facilities. Torane and Shastri (2008) who recommended the discontinued use of rapid immunochromatographic assays in donor blood screening suggested lack of sensitivity by rapid test may be due to inadequate coating of the antigens, the nature of the antigens used.and genetic heterogeneity of the virus. The study also revealed that in the case where the same type of assay brand were being used in different laboratories, varied degrees of biases were obtained as was evident in the case of DiaSpot which showed a perfect agreement with the ELISA in the Central region, but failed to detect all HBsAg positive samples in both Brong Ahafo and Ashanti regions. This was also true for Clinotech brand whose sensitivity was comparable to the ELISA in Greater Accra, but failed to detect all HBsAg positives in the Eastern region. ViruCheck rapid test on the other hand showed different sensitivity in Upper

East and Volta regions.

Although an attempt to do an inter-laboratory comparison of biases would be limited because different samples were used in each facility, and also the fact that performance of an assay is greatly affected by factors such as the technique and the storage of the test kits. However, the impact of lack of centralised control on standardised guideline on acquisition of test kits, coupled with a non-existence of a national policy geared toward ensuring the quality of pre-screening of HBsAg before transfusion cannot be underestimated (Allain and Lee, 2005). Again even in a facility it was revealed that different brands of the rapid latex immunochromatographic assay were used depending on what was available in the open market. It was estimated that each facility uses an average of four (4) different hepatitis B virus rapid latex immunochromatographic assays within a year, thus the inaccuracy of a low performing assay would be wide spread in different laboratories and this may contribute to the high risk of being infected with HBV through blood transfusion in sub-Sahara Africa estimated by Jayaraman et al., (2010) to be over four times the risk of HIV infection through blood transfusion.

Using the Modified Wald method (Agresti and Coull, 1998), the estimated residual risk of HBV transmission for the use of the rapid immunochromatographic assays for screening HBsAg in Ghana was 8.47%. This estimated risk percentage is similar to what Allain *et al.,* reported in 2003 (9.09%) as the risk of transmission of transfusion borne HBV to recipients under 10 years by the use of immnunochromatographic assay in Kumasi. Thus all these years not much improvement in the quality and reliability of pre-transfusion screening for HBV has been achieved since the screening regimen has not improved to date. The presence of HBeAg in the bloodstream is an indication of active HBV replication associated with large quantity of HBV-DNA in the bloodstream and therefore an enhanced infectivity (Lee, 1997; Yuen *et al.*, 2004; El-Sherif *et al.*, 2007; Kukka, 2008; Zahn *et al.*, 2008). Whilst the positivity for serum Anti-HBe is an indication of seroconversion with a low viral load and unlikely to produce a viable infectivity through transmission portals such as needle stick, sexual contact and household contact, it has a high potential of producing a viable infectivity through blood transfusion because of the large volume of viral DNA that is passed on to the recipient (Kukka, 2008).

HBV infection exhibits a broad spectrum of serologic patterns associated with HBV-DNA persistence. It is rare to find sero-positivity for HBeAg, anti-HBe nor both in the serum of subjects who are sero-negative for HBsAg (Brechot *et al.*, 2001; Allain *et al.*, 2003; Kidd-Ljunggren *et al.*, 2004). But as was evident in this study, out of the 13 samples that reacted sero-positive for HBeAg, only 3 were also sero-positive for HBsAg and out of the 51 samples that reacted sero-positive for Anti-HBe, only 7 also reacted sero-positive for HBsAg. Though rare undetectable HBsAg in the presence of detectable HBeAg has been reported and most of these cases had been attributed to escape mutants with point mutations in the preS1, preS2 and preS regions (Osiowy, 2002; Yang *et al.*, 2003). Chronic hepatitis with ongoing HBV replication in presence of anti-HBe has frequently been observed (Lai *et al.*, 1991), Kidd-Ljunggren et al.,(2004) reported that 65% of anti-HBe sero-positive patients who might be considered most likely to be non viremic had HBV viral DNA. Thus the non-testing of HBeAg/anti-HBe during HBV pre-donation screening may increase the residual risk of HBV transmission through blood transfusion in Ghana.

The study also revealed a high reactivity for Anti-HBc 68.54%, and this was consistent with the findings of Allain, (2006) who estimated that 75% of the Ghanaian population by the age of 16 tested sero-positive for Anti-HBc. Among the Anti-HBc sero-positive samples 8.82% also reacted sero-positive for Anti-HBs and these represent the proportion of donors that had cleared the virus and hence had developed immunity (Al-Mekhaizeem *et al.*, 2001), contrary evidence however suggests this accession may not be the case at all times (Yotsuyanagi *et al.*, 1998; Owiredu *et al.*, 2001; Dhawan *et al.*, 2008; Allain *et al.*, 2009). Among the isolated Anti-HBc sero-positive, two possible donor

groups exist, those chronically infected with persistence of HBV-DNA but had undetectable level of HBsAg and therefore produce viable viraemic infection, and those who had eventually recovered from the infection and no longer carry HBV-DNA but show no detectable Anti-HBs (Reesink *et al.*, 2008).

The ELISA test method could not be used in distinguishing between the two groups of Anti-HBc only positive donors mentioned in the preceding paragraph, however, Zahn et al.,(2008) estimates that the Ghanaian donor population includes 1.5% of the first group. It should be acknowledged however, that despite the indicative potential of this serological marker in identifying donors who have been naturally exposed to HBV, its practical use as a marker for pre-screening of blood in Ghana may not be adequate, essentially because of the high prevalence rate in the donor population (Reesink *et al.*, 2008). Though in estimating the residual risk of transfusion for the non testing of other HBV markers, this group (Anti-HBc sero -positivity) was not considered and was also not included in the total residual risk estimation, the high sero -positivity of Anti-HBc in this study even heightens the risk of getting transfused with HBV infectious blood unit in Ghana.

CONCLUSIONS

This study evaluated the performance of test kits used in pre-donation screening for HBV, and the efficiency of the screening strategy among donors in ten facilities in Ghana. The indication was that neither the kits in used nor the testing strategy in place now, is adequate to prevent transmission of hepatitis B virus through transfusion in Ghana due to high residual risk of transmission of HBV through blood transfusion. There is therefore an urgent need for an effective quality control system for the pre-donation screening of blood for HBV in Ghana.

REFERENCES

Aach R.D., Szmuness W., Mosley J.W., Hollinger F.B., Kahn R.A., Stevens C.E., Edwards V.M. and Werch J. (1981) Serum alanine aminotransferase of donors in relation to the risk of non-A,non-B hepatitis in recipients: the transfusion -transmitted viruses study. *N Engl J Med* 304, 989-994.

Agresti A. and Coull B.A. (1998) Approximate is better than "exact" for interval estimation of binomial proportions *The American Statistician* 52, 119-126

Al-Mekhaizeem K.A., Miriello M. and Sherker A.H. (2001) The frequency and significance of isolated hepatitis B core antibody and the suggested management of patients. *CMAJ* 165, 1063-1064.

Allain J.P. (2006) Occult hepatitis B virus infection and transfusion. *J Hepatol* 44, 617-618.

Allain J.P., Belkhiri D., Vermeulen M., Crookes R., Cable R., Amiri A., Reddy R., Bird A. and Candotti D. (2009) Characterization of occult hepatitis B virus strains in South African blood donors. *Hepatology* 49, 1868-1876.

Allain J.P., Candotti D., Soldan K., Sarkodie F., Phelps B., Giachetti C., Shyamala V., Yeboah F., Anokwa M. and Owusu-Ofori S. (2003) The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. *Blood* 101, 2419 -2425.

Allain J.P. and Lee H. (2005) Rapid tests for detection of viral markers in blood transfusion. *Expert Rev Mol Diagn* 5, 31-41.

Alter H.J. and Chalmers T.C. (1981) The HBsAg positive health worker revisited. *Hepatology* 1, 467-470.

Amidu N., Owiredu W.K.B.A., Addai-Mensah O., Alhassan A., Quaye L. and Batong B. (2010) Sero-prevalence and Risk Factors for Human Immunodeficiency Virus, Hepatitis B and C Viruses Infections among Blood Donors at the Bolgatanga Regional Hospital in Bolgatanga, Ghana. *Journal of the Ghana Science Association* 12.

Ampofo W., Nii-Trebi N., Ansah J., Abe K., Naito H., Aidoo S., Nuvor V., Brandful J., Yamamoto N. and Ofori-Adjei D. (2002) Prevalence of blood-borne infectious diseases in blood donors in Ghana. *J Clin Microbiol* 40, 3523-3525.

Brechot C., Thiers V., Kremsdorf D., Nalpas B., Pol S. and Paterlini-Brechot P. (2001) Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? . *Hepatology* 34,

194-203.

Dhawan H.K., Marwaha N., Sharma R.R., Chawla Y., Thakral B., Saluja K., Sharma S.K., Thakur M.K. and Jain A. (2008) Anti-HBc screening in Indian blood donors: still an unresolved issue. *World J Gastroenterol* 14, 5327-5330.

Donegan E., Stuart M., Niland J.C., Sacks H.S., Azen S.P., Dietrich S.L., Faucett C., Fletcher M.A., Kleinman S.H. and Operskalski E.A. (1990) Infection with human immunodeficiency virus type 1 (HIV-1) among recipients of antibody-positive blood donations. *Ann Intern Med* 113, 733-739.

El-Sherif A.M., Abou-Shady M.A., Al-Hiatmy M.A., Al-Bahrawy A.M. and Motawea E.A. (2007) Screening for hepatitis B virus infection in Egyptian blood donors negative for hepatitis B surface antigen. *Hepatol Int* 1, 469-470.

Jayaraman S., Chalabi Z., Perel P., Guerriero C. and Roberts I. (2010) The risk of transfusion-transmitted infections in sub-Saharan Africa. *Transfusion* 50, 433-442.

Kidd-Ljunggren K., Myhre E. and Blackberg J. (2004) Clinical and serological variation between patients infected with different Hepatitis B virus genotypes. *J Clin Microbiol* 42, 5837-5841.

Kukka M.C. (2008) HBV: How to Interpret Hepatitis B Antibody & Viral Tests. . *hcspFACTsheetSan Francisco, CA: Hepatitis C Support Project.*

Kumar H., Gupta P.K. and Jaiprakash M. (2007) The Role of anti-HBc IgM in Screening of Blood Donors. *MJAFI* 63, 350-352.

Lai M.E., Melis A., Mazzoleni A.P., Farci P. and Balestrieri A. (1991) Sequence analysis of hepatitis B virus genome of a new mutant of ayw subtype isolated in Sardinia. *Nucleic Acids Research* 19, 5078.

Lee W.M. (1997) Hepatitis B Virus Infection. *N Engl J Med* 337, 1733-1745.

Martinson F.E., Weigle K.A., Royce R.A., Weber D.J., Suchindran C.M. and Lemon S.M. (1998) Risk factors for horizontal transmission of hepatitis B virus in a rural district in Ghana. *Am J Epidemiol* 147, 478-487.

Niederhauser C., Schneider P., Fopp M., Ruefer A. and Levy G. (2005) Incidence of viral markers and evaluation of the estimated risk in the Swiss blood donor population from 1996 to 2003. *Euro Surveill* 10, 14-16.

Osiowy C. (2002) Sensitive Detection of HBsAg Mutants by a Gap Ligase Chain Reaction Assay *JCM* 40, 2566–2571.

Owiredu W.K., Kramvis A. and Kew M.C. (2001) Hepatitis B virus DNA in serum of healthy black African adults positive for hepatitis B surface antibody alone: possible association with recombination between genotypes A and D. *J Med Virol* 64, 441-454.

Reesink H.W., Engelfriet C.P., Henn G., Mayr W.R., Delage G., Bernier F., Krusius T., Assal A., Gallian P. and Corbi C. (2008) Occult hepatitis B infection in blood donors *Vox Sang* 94, 153-166.

Schreiber G.B., Busch M.P., Kleinman S.H. and Korelitz J.J. (1996) The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. *N Engl J Med* 334, 1685-1690.

Tagny C.T., Mbanya D., Tapko J.B. and Lefrere J.J. (2008) Blood safety in Sub-Saharan Africa: a multi-factorial problem. . *Transfusion* 48, 1256-1261.

Torane V.P. and Shastri J.S. (2008) Comparison of ELISA and rapid screening tests for the diagnosis of HIV, Hepatitis B and Hepatitis C among healthy blood donors in a tertiary care hospital in Mumai *ijmm* 2.

Yang X., Lei J.H., Zhang Y., Luo H.Y., Huang L., Zheng Y.H., Tang X.P. and Li L. (2003) A novel stop codon mutation in HBsAg gene identified in a hepatitis B virus strain associated with cryptogenic cirrhosis. *World J Gastroenterol* 9.

Yotsuyanagi H., Yasuda K., Iino S., Moriya K., Shintani Y., Fujie H., Tsutsumi T., Kimura S. and Koike K. (1998) Persistent viremia after recovery from self-limited acute hepatitis B. *Hepatology* 27, 1377-1382.

Yuen M.F., Fung S.K., Tanaka Y., Kato T., Mizokami M., Yuen J.C., Wong D.K., Yuan H.J., Sum S.M. and Chan A.O. (2004) Longitudinal study of hepatitis activity and viral replication before and after HBeAg seroconversion in chronic hepatitis B patients infected with genotypes B and C.. *J Clin Microbiol* 42, 5036-5040.

Zahn A., Li C., Danso K., Candotti D., Owusu-Ofori S., Temple J. and Allain J.P. (2008) Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. *J Gen Virol* 89, 409-418.

ISSN 2026-6294

Journal of Medical and Biomedical Sciences (2018) 7(2): 29-37

ORIGINAL ARTICLE

The efficacy of aqueous and ethanolic leaf extracts of *Pistia stratiotes* linn in the management of arthritis and fever

S. Kyei[1], G. A. Koffuor[1] and J. N. Boampong[2]

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana; [2]Department of Human Biology, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana.

Arthritic pain and disability are at or near the top of the list of reasons adult patients seek medical attention. This study therefore attempts to establish the efficacy of an aqueous and ethanolic leaf extract of *Pistia stratiotes* Linn (Araceae) in a rodent experimental model of arthritis and fever to ascertain its importance in the traditional management of this inflammatory disorder. The aqueous and ethanolic extracts of *P. stratiotes* at doses of 30, 100, and 300 mg/kg as well as 0.3 mg/kg methotrexate, 0.46 mg/kg diclofenac and 1 mg/kg dexamethasone were administered to formalin-induced arthritic rats. The same doses of the extracts in comparison to 150 mg/kg acetaminophen were also administered to rats in which fever had been induced with lipopolysaccharides. Data obtained was analyzed using GraphPad Prism 5.0. The results obtained indicated significant reduction ($P \leq 0.05$-0.01) in paw thickness of formalin-induced arthritic animals treated with both aqueous and ethanolic leaf extracts with effects comparable to that of methotrexate, diclofenac, and dexamethasone. Lipopolysacharride-induced fever in rats was also significantly reduced ($P \leq 0.05$-0.01) at all dose levels of aqueous and ethanolic treated animals in a manner similar to that of acetaminophen. The aqueous and ethanolic leaf extracts of *P. stratiotes* have anti-arthritic and antipyretic effect in formalin-induced arthritis and LPS-induced fever in Sprague-Dawley rats.
Journal of Medical and Biomedical Sciences (2018) 7(2), 29-37

Keywords*:* Formaldehyde-induced arthritis, Fever, Lipopolysaccharide, Prostaglandin

INTRODUCTION

Arthritis is a form of joint disorder that involves inflammation of one or more joints characterized by varied levels of pain, swelling, joint stiffness, and sometimes a constant ache around the joint(s) (CDC, 2011). The world prevalence of arthritis is estimated be around 0.3–1.2 % (Silman and Horchberg, 1993). At least 47.8 million US residents have arthritis. In Europe, the magnitude of the problem is similar, affecting 8 million in the United Kingdom and 108 million across the continent (VanItallie, 2010). The story is no different in Africa. It is the number one disabling disease in South Africa; affecting an estimated one in every seven people. About 132 Million East Africans have minimal rheumatological care (Arthritis Africa, 2012). Although there is no study on the prevalence of arthritis in Ghana the situation is projected to be no better (WHO, 2000). Arthritis makes it very difficult for individuals to be physically active and many become home bound. Arthritic pain and disability are at or near the top of the list of reasons adult patients seek medical attention. Arthritis makes it very difficult for individuals to be physically active and many become home bound.

Although non-steriodal anti-inflammatory agents remains the mainstay treatment for this degenerative inflammatory disorder, its prolong clinical use elicits numerous side effects, notable amongst them are gastric erosion, ulceration, hemorrhage, bronchospasm, kidney and live dysfunction (Lin *et al.,* 2004). Studies have shown that asymptomatic mu-

Correspondence: *G.A. Koffuor, Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. E*-mail: gkoffuor@yahoo.com

cosal damage is initially evident in 80 % of subjects after non-steroidal anti-inflammatory drug (NSAID) therapy (Ehsanullah *et al.,* 1988) however, upon continuous use of NSAIDs 15-20% of treated patients develop ulcer (Singh *et al*., 1996), and 1-3% received hospital treatment for gastrointestinal, (GI) bleeding or perforation. Fever is one of the most prominent systemic manifestations of acute inflammation, especially when an inflammation is associated with infection (Romanovsky *et al*., 2005).

A population based study projects a phenomenal increase in the consumption of non-steroidal anti-rheumatic drug from 380 million to 600 million consumers in the next two decades among the geriatric population alone (Steineyer, 2000). This consolidates the need for an urgent search for new safer and efficacious anti-inflammatory agents. One medicinal plant commonly used traditionally for inflammatory disorders is *Pistia stratiotes*. Commonly known as water lettuce or water cabbage, it is an aquatic plant, stoloniferous, floating on lakes, streams, and stagnant water ponds. It is distributed in the tropical and subtropical region of Asia, Africa, and America. (Arber, 2002). Several medicinal prowesses have been ascribed to this plant. These include anti-helmintic, anti -microbial and anti-fungal properties (Prem kumar and Shyamsundar, 2005; Achola and Indalo, 1997; Sundeep Kumar *et al.,* 2000). The anti-inflammatory and anti-pyretic activity of ethanolic extract of *P. stratiotes* has been demonstrated using carrageenan, cotton-pellet-induced granuloma model and brewer's yeast fever model (Sundeep Kumar *et al.,* 20011).

This study therefore attempts to establish the efficacy of an aqueous and ethanolic leaf extracts of *Pistia stratiotes* in a rodent formalin-induced model of arthritis as it closely depict corresponding human disease state (Greenwald, 1991) and lipopolysacharides-fever model to enhance understanding into the possible mechanism of action. This is to ascertain its importance in the traditional management of inflammatory disorders and to predict its possible mechanism in curbing fever commonly associated with inflammation (Abbiw, 1990).

MATERIALS AND METHODS

Plant Collection

Pistia stratiotes was collected from the Fosu lagoon, Cape Coast in the Central Region of Ghana (5°7' N &1°16' W) in December 2010. It was identified and authenticated by Mr. G H Sam of the Department of Herbal Medicine, CHS, KNUST, where a voucher specimen bearing the number KNUST/ HM1/11/W002 has been deposited at the herbarium for future reference.

Preparation of Extracts

The leaves of *P. stratiotes* were washed thoroughly with tap water and sun-dried. The dry leaves were milled into coarse powder by a hammer mill (Schutte Buffalo, New York, USA). In preparing the aqueous leaf extract of *P. stratiotes*, 700 g of the leaf powder was mixed with 1litre of water. The mixture was maintained at 80 ^{o}C (in a round bottom flask fitted with a reflux condenser) in a thermostatically controlled water bath for 24 h and then filtered. The filtrate was freeze dried with a Hull freeze dryer /lyophilizer 140 SQ FT (model 140FS275C, USA) into powder (percentage yield 4.7%) and stored at a temperature of 4 ^{o}C in a refrigerator. This powder was reconstituted in normal saline to a desired concentration and labeled as AQ PSE for dosing in this study. Similarly, 700 g of the leaf powder was soaked with one liter of 70 % ethanol at room temperature (27-29 ^{o}C) for 72 h and filtered. The filtrate obtained was freeze-dried into powder (percentage yield 5.2 %). Quantities of this powder was reconstituted in normal saline at desired concentrations to be referred to and used in this study as the ethanolic leaf extract of *P. stratiotes* or ET PSE.

Drugs and Chemicals

Formaldehyde (Yash Chemicals, India) was used to induce arthritis while LPS (Sigma-Aldrich, USA) was used to induce pyrexia. Diclofenac sodium (KRKA, d.d., Novo mesto, Solvenia), dexamethasone sodium (Anhui Medihel Co. Ltd), and methotrexate sodium (Dabur Pharma, New Delhi, India) were the reference anti-inflammatory agent in this study. Acetaminophen (Simpex Pharamchem Inc. USA) was the reference antipyretic.

Preparation of Reference Drugs
The reference anti-inflammatory drugs were dissolved in normal saline for the study. The drugs were freshly prepared as follows 0.3 mg/kg methotrexate, 0.46 mg/kg, 1 mg/kg dexamethasone which was administered in volumes not exceeding 10 ml/kg.

Animals
Six to eight-week old Sprague Dawley rats of either sex (180-200 g) purchased from the Centre for Scientific Research into Plant Medicine (CSIRPM), Mampong-Akwapim, Ghana, were maintained in the Animal House of Department of Pharmacology, KNUST, Ghana. The animals were housed in polyacrylic cages (34cm × 47cm× 18cm) with soft wood shaving as bedding, under ambient laboratory conditions (temperature 28 ± 2°C, relative humidity 60-70 %, and normal light-dark cycle). Females were non-pregnant. They were fed with normal commercial pellet diet (GAFCO, Tema) water *ad libitum*. All procedures and techniques used in these studies were in accordance with the National Institute of Health for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

Preliminary Phytochemical Screening
Screening was performed on AQ PSE and ET PSE to ascertain the presence of phytochemicals using standard procedures described by Wagner and Bladt (1996), Glasl (1983), Harborne (1998), and Kujur *et al.,* (2010).

Formaldehyde-Induced Arthritis and Treatment
The test was performed according to the technique developed by Brownlee in 1950. Pedal inflammation was induced by injecting 0.1 ml of 4 % formalin solution below the plantar aponeurosis of the right hind paw of the rats after measuring their paw thickness. The arthritic animals were divided into ten groups of five and treated with either 30, 100, or 300 mg/kg AQ PSE or ET PSE, orally 30 minutes after intra-plantar injection with formalin on day 1, and then daily), 0.3 mg/kg methotrexate intraperitoneally (i.p every four days), 0.46 mg/kg diclofenac (i.p, daily), 1 mg/kg dexamethasone (i.p, every other day), 1 ml/kg normal saline (p.o, daily), the control, over the experimental period.

Lipopolysaccharide-Induced Fever and Treatment
The method of Santos and Rao (1998) was modified and used for the assessment of the anti-pyretic activity of the aqueous and ethanolic extracts of *P. stratiotes*. Animals were fasted overnight prior to induction of fever, but given water *ad libitum*. Rectal temperature was measured using a lubricated ECT-1 digital thermometer (Estar Electronic And Instrument Co., Ltd., ZheJiang, China) inserted 3cm deep into the rectum of the rats. Fever was induced by injecting intramuscularly, 1 mg/kg of LPS into the right thigh of each rat. Rectal temperature was measured again and animals that showed an increase in temperature of 0.5 ºC and more were selected for the study. The animals with fever were put into eight groups of five and were treated with either 30, 100, or 300 mg/kg AQ PSE or ET PSE, 150 mg/kg acetaminophen, or 1 ml/kg normal saline solution (the control), orally, two hours after LPS-induced fever. Rectal temperature was measured at 1 h intervals for 6h. All experiments were carried out between 08.00 h and 18.00 h in a quiet laboratory with an ambient temperature of 25 ± 2ºC.

Statistical Analysis
Results were analyzed using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparisons test by using GraphPad Prism; version 5.03. Values were expressed as mean ± SEM and P values ≤ 0.05 were considered statistically significant.

RESULTS

Preliminary Phytochemical Screening
Results for the initial phytochemical screening are as shown in Table 1.

Formalin-induced Arthritis
There were significant reductions (P ≤ 0.01-0.001) in paw thickness of formalin-induced arthritic ani-

Table 1: Results of phytochemical screening of the aqueous and ethanolic extracts of *P. stratiotes* Linn

Components	AQ PSE	ET PSE
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	-
Sterols	+	+
Glycosides	+	+
Saponins	-	-
Triterpenoids	-	-

"+" implies present, "-"implies absent

mals treated with both aqueous and ethanolic leaf extracts of *P. stratiotes* compared to the normal saline -treated arthritic animals. Similar significant reductions ($P \leq 0.001$) in paw thicknesses were observed among the methotrexate, diclofenac, and dexamethasone treated arthritic animals (Figure 1, 2 and 3).

Lipopolysacharride –Induced Fever

Lipopolysacharride- induced fever in rats was significantly reduced ($P \leq 0.01$-0.001) at all dose levels of AQ PSE and ET PSE treatment; the effect was similar to that observed for acetaminophen treatment (Figures 4 and 5).

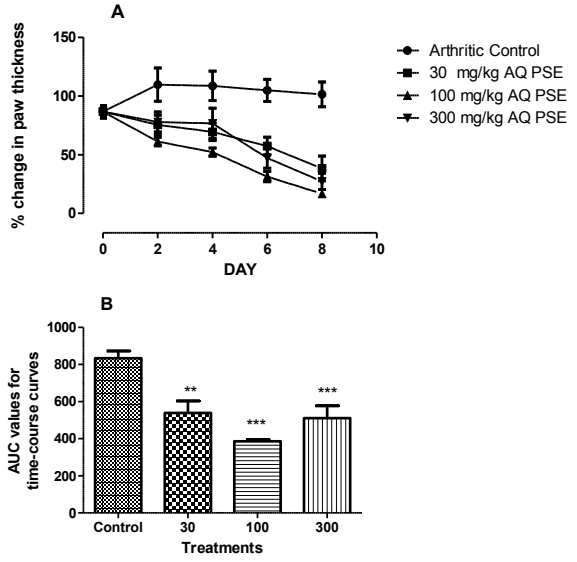


Figure 1: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of AQ PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). ** implies $P \leq 0.01$, ***implies $P \leq 0.001$: the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in paw thickness was computed using the formula V=(Vt-Vo)/ Vo × 100 where V is percentage in paw thickness, Vt is paw thickness after formalin challenge, Vo is the initial paw thickness before formalin challenge

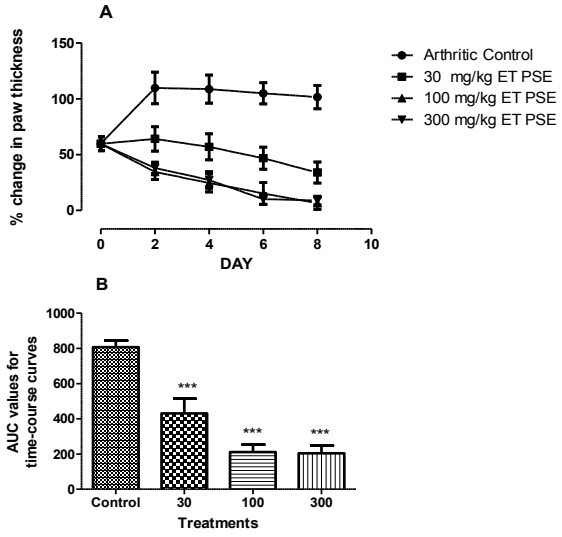


Figure 2: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of ET PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). * implies $P \leq 0.001$; the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in paw thickness was computed using the formula V=(Vt-Vo)/ Vo × 100 where V is percentage in paw thickness, Vt is paw thickness after formalin challenge, Vo is the initial paw thickness before formalin challenge**

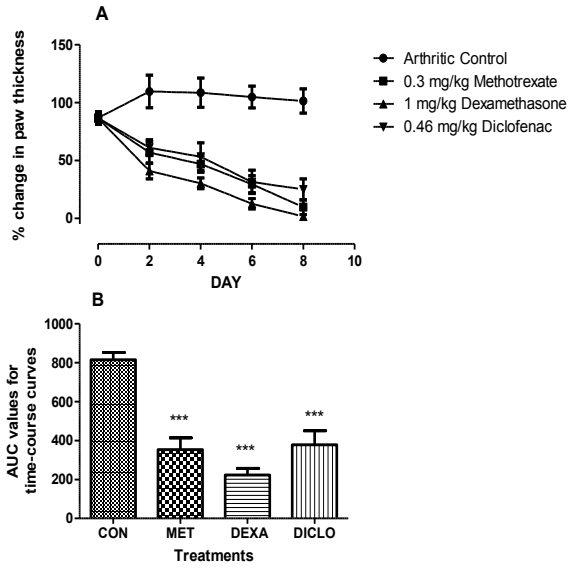


Figure 3: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 0.3 mg/kg methotrexate, 1 mg/kg dexamethasone, and 0.46 mg/kg diclofenac on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). * implies $P \leq 0.001$; the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in paw thickness was computed using the formula V=(Vt-Vo)/ Vo × 100 where V is percentage in paw thickness, Vt is paw thickness after formalin challenge, Vo is the initial paw thickness before formalin challenge**

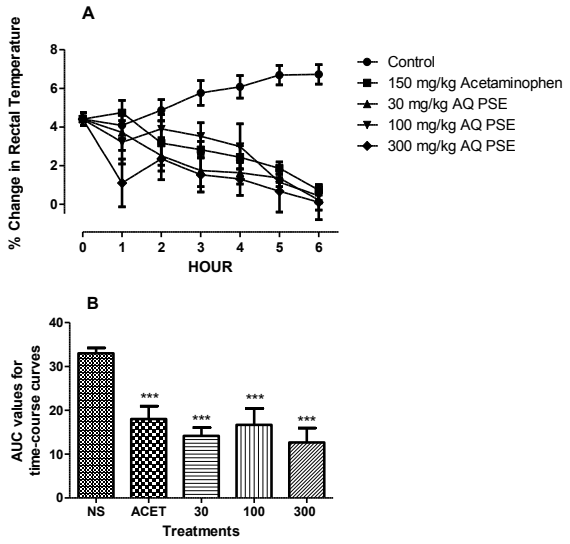


Figure 4: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of AQ PSE and 150 mg/kg acetaminophen on LPS-induced fever in Sprague-Dawley rats. Data plotted are means ± SEM (n=5). ***implies $P \leq 0.001$; the level of significance of rectal temperature reduction (compared to the normal saline-treated) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in rectal temperature was computed using the formula T=(Tt-To)/ To × 100 where T is percentage in rectal temperature, Tt is rectal temperature after LPS challenge, To is the initial rectal temperature before LPS challenge

DISCUSSION

It is established that inhibition of formalin-induced paw oedema in rats is one of the most appropriate modus operandi to screen for anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis (Greenwald, 1991). Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response (Wheeler-Aceto and Cowan, 1991). Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation (Banerjee *et al.,* 2000).

The reference drugs and both extracts of *P. stratiotes* significantly suppressed formalin-induced arthritis. Dexamethasone is acknowledged to inhibit the release of pro-inflammatory cytokines (TNF-α, Tumor Necrosis Factor- α and IL-1β, interleukin-1β), which are known to play a central role in the prop-

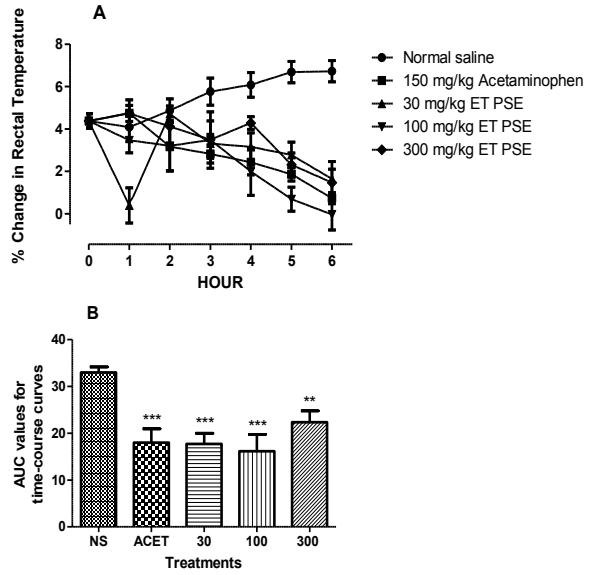


Figure 4: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of ET PSE and 150 mg/kg acetaminophen on LPS-induced fever in Sprague-Dawley rats. Data plotted are means ± SEM (n=5). ** implies P ≤ 0.01; ***implies *P* ≤ 0.001: the level of significance of rectal temperature reduction (compared to the normal saline-treated) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in rectal temperature was computed using the formula T=(Tt-To)/ To × 100 where T is percentage in rectal temperature, Tt is rectal temperature after LPS challenge, To is the initial rectal temperature before LPS challenge

agation of the disease process in arthritis thus its ability to arrest paw swelling (Issekutz and Issekutz, 1991). Methotrexate inhibits proliferation of the lymphocytes and other cells responsible for inflammation in the joint (Gubner, 1951). The anti-inflammatory effect of diclofenac is mediated mainly through inhibition of COX and prostaglandin production (Furst and Manning, 2001).

Fever is one of the most prominent systemic manifestations of acute inflammation, especially when an inflammation is associated with infection (Romanovsky *et al.*, 2005). These reactions represent the primary host defense response to infection; collectively called the "acute-phase reaction"(Blatteis, 1992). The usual view of the mechanism by which infectious fevers are produced stipulates that infectious noxa e.g. bacterial endotoxic lipopolysaccharides (LPS) that invade the body activate mononuclear phagocytes that then produce and release pyrogenic cytokines including IL-1 β and TNF-α. These are transported via the bloodstream to the ventromedial preoptic area of the anterior hypothalamus, the "fever producing center", where they operate (Saper, 1998; Roth and De Souza, 2001; Dunn, 2002; Dinarello, 2004). It is, however, doubtful how cytokines, as hydrophilic peptides, could penetrate the brain. That is to say, it is generally believed that, rather than acting directly, the cytokines induce the local generation and release of prostaglandin E_2, a lipid mediator that is obviously thermogenic when injected centrally (Blatteis, 1997; Ivanov and Romanovsky, 2004). Its production is dependent on the activation of two enzymes, cyclooxygenase (COX)-2 and microsomal PGE synthase-1, which catalyze its conversion from arachidonic acid present in the membranes of cells (Ivanov *et al.,* 2002).

Acetaminophen is a reputable antipyretic analgesic agent, often administered therapeutically to ease pain and fever (Ayoub *et al.,* 2004). The main mechanism proposed is the inhibition of COX, and recent findings suggest that it is highly selective for COX-2 (Hinz *et al.,* 2008). Paracetamol reduces the oxidized form of the COX enzyme, preventing it from forming pro-inflammatory chemicals (Roberts *et al.,* 2001; Högestätt *et al.,* 2005).This leads to a reduced amount of Prostaglandin E2 in the CNS, thus lowering the hypothalamic set-point in the thermoregulatory centre.

Oral administration of both aqueous and ethanolic leaf extracts of *P. stratiotes* as earlier indicated could possibly be inhibiting COX-2 and subsequent production of prostaglandins thereby exhibiting potent hypothermic effect in LPS-induced fever in Sprague -Dawley rats at much lower doses. (Sundeep Kumar

et al., 2011).

The presence of biologically active phytochemicals present in both the aqueous and ethanolic extracts of *P. stratiotes* could have contributed to the anti-inflammatory activity. Tannins (Mota *et al.,* 1985; Owoyele *et al.,* 2010), flavonoids (Borissova *et al.,* 1994; Hämäläinen *et al.,* 2007), sterols (Bouic *et al.,* 1996; Bouic, 1998; Akihisa *et al.,* 2007), alkaloids (Barbosa-Filho *et al.,* 2006) and glycosides (Odontuya *et al.,* 2005; Liu and Wang, 2011) have been documented to have anti-inflammatory effect via several mechanisms.

CONCLUSION

P. stratiotes has anti-arthritic and antipyretic effect in formalin-induced arthritis and LPS-induced fever in Sprague-Dawley rats.

ACKNOWLEDGEMENT

The Authors are grateful for the technical assistance offered by Mr. Thomas Ansah of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana for his technical assistance and Mr. G H Sam of the Department of Herbal Medicine, CHS, KNUST, Kumasi, Ghana for identification and authentication of the plant.

REFERENCES

Abbiw DK (1990).Useful plants of Ghana: West African uses of wild and cultivated plants. Intermediate Technology Publications and the Royal Botanic Gardens Kew.

Achola KJ, Indalo AA. Pharmacologic activities of *Pistia stratiotes* (1997). *Pharmaceut Biol 35(5): 329-333.*

Akihisa T, Nakamura Y, Tagata M, Tokuda H, Yasukawa K, Uchiyama E, Suzuki T, Kimura Y (2007).Anti-inflammatory and anti-tumor-promoting effects of triterpene acids and sterols from the fungus Ganoderma lucidum. *Chem Biodivers. 4(2):224-31.*

Arber A (2002). The Vegetative Morphology of Pistia and the Lemnaceae*Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character.* 91, :636 pp. 96-103 The Royal Society .Available from URL: http://www.jstor.org/stable/80788

Ayoub SS, Botting RM, Goorha S, Colville-Nash PR, Willoughby DA, Ballou LR (2004). Acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein. *Proc Natl Acad of Sci USA 101:30;11165–11169*

Banerjee S , Kumar Sur T, Mandal S, Chandra Das P, Sikdar S (2000). Assessment of the anti-inflammatory effects of *Swertia chirata* in acute and chronic experimental models in male albino rats. *Indian Journal of Pharmacol 32: 21-24*

Barbosa-Filho JM, Piuvezam MR, Moura MD, Silva MS, Batista Lima KV, Leitão da-Cunha EV Fechine IM, Takemura OS (2006).Anti-inflammatory activity of alkaloids: a twenty-century review .*Rev. bras. farmacogn. 16 (1)*

Blatteis CM (1992). The pyrogenic action of cytokines Interleukin-1 in the Brain. Oxford: Pergamon Press; 93–114.114eds. Rothwell NJ and Dantzer RD.

Blatteis CM. (1997) Prostaglandin E2: a putative fever mediator. In: Mackowiak P.A. (Ed.), *Fever: Basic Mechanisms and Management* (2nd ed.). Lippincott-Raven, Philadelphia, PA, pp. 117–145

Borissova P, Valcheva S, Belcheva A(1994). Anti-inflammatory effect of flavonoids in the natural juice from Aronia melanocarpa, rutin and rutin-magnesium complex on an experimental model of inflammation induced by histamine and serotonin. *Acta Physiol Pharmacol Bulg. 20(1):25-30.*

Bouic PJD (1998). Sterols/Sterolins: The natural, nontoxic immuno-modulators and their role in the control of rheumatoid arthritis. *The Arthritis Trust;Summer:3-6.*

Bouic PJD, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP(1996).Beta-sitosterol and beta-sitosterol glycoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. *Int J Immunopharmacol 18:693-700.*

Brownlee G. (1950). Effect of deoxycortone and

ascorbic acid on formaldehyde induced arthritis in normal and adrenalectomised rats. *Lancet. 1, 157-l 59*

Centre of Disease Control and Prevention. Arthritis Basics. Division of adult and community health, National Centre for Chronic Disease Prevention and Health Promotion (2011).

Dinarello CA. (2004) Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J. Endotoxin Res., 10: 201–222.*

Dunn AJ (2002). Mechanisms by which cytokines signal the brain. *Int. Rev. Neurobiol., 52: 43–65.*

Ehsanullah RS, Page MC, Tildesley G, Wood JR (1988). Prevention of gastroduodenal damage induced by non-steroidal anti-inflammatory drugs: controlled trial of ranitidine. *Br Med J 297:1017-21.*

Furst DE, Manning DC (2001). Future directions in pain management. *Clin. Exp. Rheumatol. 19:71-76.*

Glasl H (1983). Zur Photometrie in der Drogenstandiserung. DAZ, 123:1979-1987

Greenwald RA (1991). Animal models for evaluation of arthritic drugs. *Meth Find Clin Pharmacol;**13**:75-83.*

Gubner R (1951). Effect of aminopterin on epithelial tissues. *Arch. Dermatol. 64, 688-699.*

Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E (2007). Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators Inflamm. 2007:45673.*

Harborne JB (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd ed. London. Chapman and Hall. p 302

Hinz B, Cheremina O, Brune K (2008). Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. *The FASEB 22 (2): 383–390.*

Högestätt ED, Jönsson BA, Ermund A, Andersson DA, Björk H, Alexander JP, Benjamin F. Cravatt BF, Basbaum AI and Zygmunt PM (2005). Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *J. Biol. Chem. 280 (36): 31405–12.*

Issekutz AC. and Issekutz TB (1991). Quantitation and kinetics of polymorphonuclear leukocyte and lymphocyte accumulation in joints during adjuvant arthritis in the rat. *Lab. Invest., 64: 656-663.*

Ivanov AI and Romanovsky AA (2004). Prostaglandin E2 as a mediator of fever: synthesis and catabolism. *Front. Biosci.9: 1977–1993.*

Ivanov AI, Pero RS, Scheck AC, Romanovsky AA (2002). Prostaglandin E (2)-synthesizing enzymes in fever: differential transcriptional regulation. *Am. J. Physiol., 283: R1104–R1117*

Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S, Roy BK (2010). Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. *Phcog Res 2:258-63*

Lin J, Zhang W, Jones A, Doherty M (2004). Efficacy of topical non-steroidal anti-inflammatory drugs in the treatment of osteoarthritis: meta-analysis of randomised controlled trials. *Br Med J;329:324*

Liu X, Wang J. Anti-inflammatory effects of iridoid glycosides fraction of Folium syringae leaves on TNBS-induced colitis in rats (2011). *J Ethnopharmacol. 27;133(2):780-7.*

Mota ML, Thomas G, Barbosa Filho JM (1985). Anti-inflammatory actions of tannins isolated from the bark of Anacardium occidentale L. *J Ethnopharmacol. 13(3):289-300.*

National Institutes of Health, Office of Science and Health Reports,Guide for care and use of laboratory animals. Publication no. 83-23.Office of Science and Health Reports, Department of Health and Human Services. Bethesda, 1996 MD

Odontuya G, Hoult JRS, Houghton PJ (2005).

Structure-Activity Relationship for Anti-inflammatory Effect of Luteolin and its Derived Glycosides. *Phytother. Res. 19, 782–786*

Owoyele BV, Negedu MN, Olaniran SO, Onasanwo SA, Oguntoye SO, Sanya JO Oyeleke SA, Ibidapo AJ, Soladoye AO (2010). Analgesic and anti-inflammatory effects of aqueous extract of Zea mays husk in male Wistar rats. *J Med Food. 13(2):343-7*.

Prem kumar VG, Shyamsundar D. Antidermatophytic activity of pistia stratiotes (2005). *Indian J Pharmacol.37:127-8*

Roberts LJ, Marrow, J.D (2001). "Analgesic-antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout" in, "Goodman & Gilman's The Pharmacological Basis of Therapeutics 10th Edition" by Hardman, J.G. & Limbird, L.E. Published by McGraw Hill. pp.687–731

Romanovsky AA, Almeida MC, Aronoff DM, Ivanov AI, Konsman JP, Steiner AA, Turek VF (2005). Fever and hypothermia in systemic inflammation: Recent discoveries and revisions. *Front. Biosci. 10: 2193-2216*

Roth J, De Souza, GE (2001). Fever induction pathways: evidence from responses to systemic or local cytokine formation. *Braz. J. Med. Biol. Res., 34: 301–314*.

Santos FA, Rao VS (1998). A study of the antipyretic effect of quinine, an alkaloid effective against cerebral malaria, on fever induced by bacterial endotoxin and yeast in rats. *J Pharm Pharmacol 50: 225-9*.

Saper CB (1998). Neurobiological basis of fever. *Ann. N.Y. Acad. Sci., 856: 90–94*.

Silman AJ, Horchberg MC (1993). Rheumatoid arthritis In: Silman AJ, Horchberg MC eds. Epidemiology of the rheumatic diseases. Oxford: Oxford Medical Publications, 7–*68*.

Singh G, Ramey DR, Morfeld D, Shi H, Hatoum HT, Fries JF (1996). Gastrointestinal tract complications of nonsteroidal anti-inflammatory drug treatment in rheumatoid arthritis: a prospective observational cohort study. *Arch Intern Med. 156(14):1530-6*.

Steinmeyer J (2000).Die medikamentöse Therapie der Arthrose. *Sport Orthop Traumatol **16**:19-25*

Sundeep Kumar HK, Anindya Bose, Arundhuti Raut, Sujit Kumar Sahu S and Raju M. B. V (2000)**.** Evaluation of Anthelmintic Activity of *Pistia stratiotes* Linn. *J Basic and Clin Pharm, JBCP 1: (2) (2000)*

Sundeep Kumar HK, Raju MBV, Dinda SC, Sahu SK and Banerjee M (2011). Analgesic, Anti-inflammatory and Antipyretic Activity of *Pistia Stratiotes* L. *RASAYAN J. Chem.4 (3) ;506-511*

Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography. 2nd ed. New York. Springer Verlag, 1996

Wheeler-Aceto H, Cowan A (1991). Neurogenic and tissue mediated components of formalin-induced oedema. *Agents Actions;**34**:264-*9.

WHO (2003). The burden of Musculoskeletal conditions at the start of the new millennium. Report of a WHO Scientific Group WHO Technical Report Series 919. Geneva

ISSN 2026-6294