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ORIGINAL ARTICLE

Thyroid Disorders in Accra, Ghana: A Retrospective Histopathological Study at the Korle-Bu Teaching Hospital

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There is a scarcity of data on thyroid disorders in Ghana. This retrospective study examined the spectrum and incidence of thyroid disorders by reviewing all thyroid disorders reported in the Department of Pathology, Korle-Bu Teaching Hospital (KBTH) between 2004 and 2010. Data was collected on the clinical and histological characteristics of all thyroid disorders reported during the study. 1300 (3.7%) cases were reported, representing an annual incidence of 185.7 cases. The ages ranged from 1-86 years with a mean of 41.5 (SD=13.9). Most [353 (27.4%)] of the cases were between 30-39 years group. Majority, 1141(87.8%) were females. The top eight common thyroid diseases were; non-toxic multinodular goitre 1002(77.5%), follicular adenoma 86(6.6%), diffuse toxic goitre 42 (3.2%), papillary thyroid carcinoma 40(3.1%), thyroglossal duct cyst 35(2.7%), Hashimoto's thyroiditis 28(2.2%), lymphocytic thyroiditis 22(1.7%) and follicular carcinoma 17(1.3%). Sixty-six (43.4%) of the neoplastic thyroid disorders were malignant with a prevalence of 0.18 among thyroid samples and annual incidence of 9.40 cases. The commonest thyroid cancer was papillary carcinoma 40(60.6 %), with a mean age of 38.3 SD=16.1 years, majority, 34 (82.9%), were women. A wide spectrum of thyroid disorders exists in Ghana, with an annual incidence of 185.7 cases. The commonest malignant thyroid disorder was papillary carcinoma, though iodine deficiency is endemic in Ghana and on this basis; one would have expected follicular carcinoma to be the commonest thyroid cancer in Ghana.

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Keywords*:* Multinodular; Goitre; Papillary; Follicular; Adenoma, Ghana

INTRODUCTION

Thyroid disorders vary according to the geographic location, environmental factors, major radionuclear events, factors affecting the onset and persistence of iodine-deficiency as well as iodine excess in diet and the population studied (Gheriani, 2006). The prevalence of thyroid disorders has been found to increase linearly with age (Mariotti *et al.*, 1995), and virtually all thyroid diseases are common in women, who commonly present with palpable anterior neck swelling (Tunbridge *et al.*, 1977; Vander *et al.*, 1968).

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Disorders of the thyroid gland are grouped into hyperplasia, neoplasia, thyroiditis and developmental (Hedinger *et al.*, 1989; Rosai *et al.*, 1992). Studies outside Ghana have identified non-toxic multinodular goitre as the commonest thyroid disorder especially amongst females (Ogbera and Kuku, 2011; Santaniello *et al.*, 2012). Follicular adenoma is found in most studies to be the most common neoplastic thyroid tumour (Meissner and Warren, 1969). Data on the frequency of thyroid malignancies is not conclusive. Some studies in Africa have found papillary carcinoma to be the commonest thyroid malignancy (Baloch and LiVolsi, 2002; Thomas and Ogunbiyi, 1995) whilst others discovered follicular carcinoma to the commonest (Edino *et al.*, 2010;

Kalk *et al.*, 1997; Rahman *et al.*, 2010).

Recent studies have established that iodization of food and water resulted in a reduction in the incidence of follicular neoplasms and anaplastic cancers whilst cases of papillary cancers, medullary cancers, primary thyroid lymphomas and lymphocytic thyroiditis are on the increase (Harach and Ceballos, 2008). Among the known inflammatory thyroid disorders, Hashimoto's thyroiditis has been identified as the commonest especially in females (Okayasu *et al.*, 1994). Although thyroid disorders are numbered among the common endocrine disorders in Africa (Ogbera and Kuku, 2011), the spectrum and prevalence of thyroid diseases have not been studied in Ghana. The objective of this study was to determine the spectrum and prevalence of thyroid disorders using retrospective histopathological data from the Department of Pathology Korle-Bu Teaching Hospital.

MATERIALS AND METHODS

Study Site and study design

All data were gathered from the Department of Pathology, affiliated to the University of Ghana Medical School, and the largest in the country, Ghana, which reports between 5,000 and 8,000 histological cases in a year. This Department receives surgical specimens from Korle-Bu Teaching Hospital, the largest referral hospital in Ghana; as well as cases within the Accra Metropolis, neighboring towns and Districts, and other regions such as the Central, Western Eastern and Volta regions of Ghana. Although this retrospective study was a single-institution experience, a sample that will allow for evaluation of the clinical and histological characteristics of thyroid disorders in Ghana was used.

Sampling techniques and sample size

All the histopathology request forms and slides of confirmed thyroid disorders received in the Department of Pathology from January 2004 through December 2010 were reviewed independently by two pathologists, for clinical characteristics like age, main complaint and duration as well as histological features. A total of 1300 thyroid cases were reviewed during the study period. Branchial pouch anomalies, epidermoid cyst and all poorly fixed specimens were excluded in the study.

Classification of thyroid disorders

In this study, thyroid disorders were classified into four main categories: developmental- thyroglossal duct cyst and heterotopic thyroid tissue. Hyperplasia: sub-classified into non-toxic multinodular goitre, Grave's disease (diffuse toxic goitre) and dyshormonogenetic goitre, based on their presumed mechanism of production, morphologic features and clinical manifestations. Neoplasia: traditionally divided into: benign follicular adenoma and carcinomas (papillary, follicular, insular, medullary and anaplastic). Thyroiditis was divided into autoimmune (Hashimoto's thyroiditis and Lymphocytic thyroiditis) and non-autoimmune (Granulomatous and Acute thyroiditis).

Statistical analysis

The data were entered into a computerized spreadsheet and analyzed using SPSS software (Version 18). Frequency distributions and descriptive statistics were calculated for each variable.

RESULTS

Table 1 shows the clinico-pathological characteristics of the study population. During the period of study (2004-2010), a total of 1300 (3.7%) thyroid specimens were reported in our institution, giving an annual incidence of 185.7 cases. Most [618 (47.6%)] were total thyroidectomy specimens, with 427 (32.8%) subtotal thyroidectomies. The ages of patients diagnosed with thyroid disorders ranged from 1 year to 86 years, with mean age of 41.5 (SD=14.0). Most (27.4%) of the study population were young and in the 30-39 years age group (Table 1). In all 1152 (89.6%) of study participants were below 60 years with 134 (10.4%) being 60 years and above. The ages of 14 participants were not available. Majority [1141 (87.8%)] of the study participants were females. All 1300 (100%) patients, presented with neck swelling. A total of 84 (6.5%) patients had additional complains, majority [65 (77.4%)] were toxic symptoms (palpitation, tremors) (Table 1). Three hundred and seventy-one (28.5%),

of the cases had stated duration at the time of diagnosis, of which 85(22.9%) reported within seven to twelve months of noticing the swelling. The most frequently diagnosed group of thyroid disorders were the hyperplasias, 1043(81.1%) followed by neoplasms 153 (11.9%) (Table 1). The top eight common thyroid diseases were; non-toxic multinodular goitre 1002 (77.7%), follicular adenoma 86(6.9%), diffuse toxic goitre 42(3.2), papillary thyroid carcinoma 40(3.1%), thyroglossal duct cyst 35(2.7%), Hashimoto's thyroiditis 28(2.2%), lymphocytic thyroiditis 22(1.7%) and follicular carcinoma 17(1.3%) (Table 3).

Of the 1044 cases of hyperplasia, 1002(96.0%) were non-toxic multinodular goitre with mean age 42.5 SD=13.2, most [282(28.1%)] were within the 30-39 year group. Majority [900(89.7%)] were women (Table1). All the patients presented with anterior neck swelling with degenerative changes (haemorrhage, fibrosis, cystic changes, cholesterol cleft and dystrophic calcifications). Of the 292 (29.1%) cases of non-toxic multinodular goitres who had stated duration at presentation, many 65 (22.3%) reported within seven to twelve months of noticing the swelling.

The ages of the 153(11.9%) patients diagnosed with neoplastic thyroid diseases ranged from 17 to 86 years, with a mean age of 40.6 years [SD=14.6] many [38 (24.8%)] were within the 30-39 age group. Majority 123(80.4%) of the patients were females. Of those with stated duration at presentation most [12 (24.0%)], of reported within seven to

Table1: Clinico-pathological characteristics of the study population

1. Age distribution of all thyroid disorders of thyroid cases

Range: 1-86 years; **Mean age**: 41.5 **SD**: 13.9

Age groups (years)	**Frequency (n)**	**Percentage(%)**
1-9	11	0.9
10-19	33	2.7
20-29	199	15.5
30-39	353	27.4
40-49	330	25.7
50-59	224	17.4
60 and above	103	10.5
Total	1286	100.0

2. **Gender characteristics of the study population**

Female: 1141(87.8%) **Male:** 159(12.2%)

3. **Major Clinical Presentation of Study Population**: Anterior Neck Swelling: 1300(100.0%)

4. **Additional Complains** (N=84)

Toxic symptoms 65(77.4%) ii. Pressure symptoms 17(20.2%) iii. Other symptoms 2(2.4%)

5. Duration at Presentation (N=371)

Months	1-6	7-12	13-24	25-60,	61-120	>120
N/%	75(20.2)	85(22.9)	59(15.9%)	70(18.9)	45(17.1)	37(10.0)

6. Types of Surgical Specimens(1300)

Total Thyroidectomies	618(47.6%)
Subtotal Thyroidectomies	427(32.8%)
Left Lobectomies	117(9.0%)
Right Lobectomies	79(6.1)
Excision	48(3.7%)
Others	10(0.8%)
Total	1300(100.0%)

7. Major Groups of Thyroid Disorders

Hyperplasia 1044(81.1%), Neoplasia 153(11.1%), Thyroiditis 52(4.0%), Developmental 38(3.0%)

Table 2: Demographic characteristics of the major thyroid disorders

Groups	Range	Peak age	Mean	Male (n/%)	Female n/%)
Hyperplasia (n = 1044)					
NTMG	14-85	30-39	42.5	102(10.3%)	900(89.7%)
DTG	20-59	40-49	39.3	2(4.8%)	40(95.2%)
Neoplasia (n = 152)					
Follicular adenoma	18-85	30-39	38.8	12(14.0%)	74(86.0%)
Papillary carcinoma	17-72	20-29	38.3	7(17.5%)	33(82.5%)
Follicular carcinoma	17-86	50-59	52.1	8(47.1%)	9(52.9%)
Medullary carcinoma	35-51	40-49	44.2	3(60.0%)	2(40.0%)
Others	20-69	30-39	40.1	2(50.0%)	2(50.0%)
Thyroiditis (n = 52)					
Hashimoto's	21-69	30-39	43.3	1(3.6%)	27(96.4%)
Lymphocytic	21-64	30-39	39.9	2(13.0%)	20(87.0%)
Others (2/3.8%)					
Developmental (n = 38)					
Thyroglossal duct cyst	1-60	20-29	21.1	17(48.6%)	18(51.4%)
Simple thyroid cyst (3/8.0%)					

Table 3: Demographic characteristics of the first eight common thyroid diseases

Disease	Number N (%)	Peak age (yrs)	Mean (yrs)	Male N (%)	Female N (%)
Non-toxic Multinodular Goitre	1002(77.9)	30-39	42.4	102(10.3)	900(89.7)
Follicular Adenoma	86(6.7)	30-39	38.8	12(14.0)	74(86.0)
Diffuse Toxic Goitre	42(3.2)	40-49	39.3	2(4.8)	40(95.2)
Papillary carcinoma	40(3.1%)	20-29	38.3	7(17.5)	33(82.5)
Thyroglossal Duct Cyst	35(2.7%)	20-29	21.1	17(48.6)	18(51.4)
Hashimoto's Thyroiditis	28(2.2%)	30-39	43.3	1(3.6%)	27(96.4%)
Lymphocytic Thyroiditis	22(1.7%0	30-39	39.8	2(9.1%)	20(90.9%)
Follicular carcinoma	17(1.3%)	50-59	52.1	8(47.5%)	9(52.9%)

twelve months. Benign follicular adenoma was the commonest [86(57.0%)] histologic subtype of the neoplastic thyroid disorders, with mean age of 38.8 (SD= 13.2). About 30% [25(29.1%] were within 30-39 age group. Of the 38(2.9%), of developmental lesion in this study, majority 35(98.5%) were thyroglossal duct cysts, with mean age of 21.1 years SD= 16.6, many were within the peak age group of 20-29 years (Table 2).

The major demographic characteristics of the major disorders are shown in Table 2. A total of 66 (43.4%) of the neoplastic thyroid disorders were malignant. The prevalence of thyroid malignancies in this study was 0.18, whiles the annual incidence was found to be 9.40 cases. The commonest thyroid cancer was papillary carcinoma [40(60.6 %)], followed by follicular carcinoma 18(27.3%) (Table 2). The ages of patients diagnosed with papillary carcinoma (Classic, 33 and follicular variant, 7) ranged from 17 -72 years with a mean of 38.3, SD=16.3 years, many [12 (30.0%)] were in the 20-29 year age group. Majority [34 (82.9%)], were women, who presented within 2 years after noticing the swelling. Patients with follicular carcinoma 17 (11.3%) (Classic, 15 and Hurthle cell carcinoma, 2)

ranged in ages from 17-86 years, with a mean age of 52.1, SD=15.6. Many 7(41.2%) were within 50-59 age group. There were 9(52.9%) females and 8 (47.1%) males. Three (33.3%) out of nine reported within 6 months of noticing the swelling. There were five cases of medullary thyroid carcinomas, mean age 44.2 SD=6.6 years, with majority 3(60.0%) were within the peak age group of 40-49 years. Majority 4 (80.0) were males.

A total of 52(4.0%) of the study population were diagnosed with thyroiditis, the ages ranged from 21-69 years, with mean age of 41.5 SD=12.8 years. Majority 48(92.3) were females. The commonest 28 (54.95) thyroiditis in this study was Hashimoto's thyroiditis with mean age of 43.3 SD=13.5 years, many 11(39.3%) were in the peak age group of 30-39 years. Almost all 27(96.4%) were females (Table 2). Of the 9(32.1%) patients with additional symptoms, 6 (66.7%) were toxic.

DISCUSSION

Abnormal function of the thyroid is known to have several public health implications. This study used retrospective histopathological data from the pathology department of the KBTH to describe the spectrum, prevalence and incidence of thyroid disorders. An annual incidence of 185.7 thyroid disease cases was reported in this study within the seven year period (2004-2010) that the study was conducted.

An increased prevalence of thyroid diseases has been reported in the aged (Brent, 2010). The relatively younger age of participants at diagnosis in this study is inconsistent with the findings of several studies (Cooper, 2004; Mariotti and Cambuli, 2007; Surks *et al.*, 2004). Non-toxic multinodular, follicular adenoma, diffuse toxic goitre, papillary carcinoma, thyroglossal duct cyst, Hashimotto's thyroiditis, lymphocytic thyroiditis and follicular carcinoma were the predominant thyroid disorders observed among cases reported in this study. This study confirmed the several reports of high prevalence of thyroid disorders among women (Tunbridge *et al.*, 1977; Vander *et al.*, 1968). Several studies have reported that non toxic thyroid nodules are a frequent occurrence in clinical practice and that advanced age and female gender are notable risk factors (Gharib, 1997; Leech *et al.*, 1928; Rojeski and Gharib, 1985; Tunbridge *et al.*, 1977). In this study, majority of the females with nodular goitre were relatively younger (Table 1). Iodine deficiency, which is endemic in Ghana, has been linked with the pathogenesis of multinodular goitre. Our findings are similar to studies conducted in iodine deficient regions in other parts of the World (Ogbera and Kuku, 2011; Santaniello *et al.*, 2012). One other observation in this study is that all the non-toxic multinodular goitres showed secondary changes which shows that thyroid disorders in Ghana are usually chronic.

Studies have shown that follicular adenoma is the commonest benign neoplastic thyroid disorder reported in females and second to non-toxic multinodular goitre (Meissner and Warren, 1969). Our findings confirm these reports.

Papillary carcinoma and follicular carcinoma were the commonest thyroid malignancies reported in this study. Some studies have identified papillary thyroid carcinoma as the most common thyroid cancer (Baloch and LiVolsi, 2002; Thomas and Ogunbiyi, 1995), whiles others identified follicular carcinoma to be the most common (Kalk *et al.*, 1997; Rahman *et al.*, 2010). These findings however, were with reference to the geographic location, major radioactive fall-outs and the iodine status of the population studied. Our findings, are in agreement with those studies that identified papillary carcinoma as the commonest thyroid cancer. The introduction of iodization of food and water has led to a decline in the incidence of follicular and anaplastic neoplasms, whilst papillary cancers, medullary cancers, primary thyroid lymphomas and lymphocytic thyroiditis are on the increase (Harach and Ceballos, 2008). However, this programme which has been ongoing for the last seventeen (17) years cannot be used as the basis for the high number of papillary carcinoma since there is no report to that effect.

Iodine deficiency is endemic in Ghana and it will thus be appropriate to suggest that follicular carcinoma should be the commonest thyroid malignan-

cy, knowing the link that exists between iodine deficiency and follicular lesions. However, this wasn't the case in this study. In view of this, we recommend a before and after study on the spectrum of thyroid malignancies in Ghana. This will enable a proper evaluation of thyroid malignancies in Ghana.

In this study follicular carcinoma was diagnosed in relatively older patients in contrast to publications that have associated papillary thyroid cancinoma with the elderly (Lin *et al.*, 2004; Schlumberger, 1998). Furthermore, both papillary and follicular cancers showed female predominance, however we observed that follicular carcinoma was relatively common in males. On the contrary, almost all of the five cases of medullary carcinomas were males whose ages were similar to the mean age of the study participants.

The commonest thyroiditis in this study was Hashimoto's thyroiditis with a peak age group of 30-39 years and mean age 43.3 years and tended to be more prevalent in females. This is consistent with reports in other studies with the exception of age for which participants in this study with Hashimoto's thyroiditis were younger than the age reported by Contran *et al.,* (1994). Similarly, chronic lymphocytic thyroiditis had a peak age and gender distribution similar to that of Hashimotos's thyroiditis, but study participants with the condition were relatively younger.

CONCLUSION

A wide spectrum of thyroid disorders exists in Ghana, with an annual incidence of 185.7 cases. The commonest thyroid cancer in our study is papillary thyroid carcinoma, contrary to the fact iodine deficiency is endemic in Ghana and on this basis, one would have expected follicular to be the commonest thyroid cancer in Ghana**.** A population based study of the prevalence of thyroid diseases in Ghana is strongly indicated. Secondly, a retrospective study which will enable a proper evaluation of thyroid malignancies in Ghana with regards to the national iodization program (before and after) is further recommended.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Antipyretic activity of Polyalthia longifolia Benth. & Hook. F. var. pendula (Annonaceae), on lipopolysaccharide-induced fever in rats

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Fever is a complex physiological response triggered by infectious or aseptic stimuli. The present investigation was carried out to study the antipyretic activity of *Polyalthia longifolia* extracts in Wistar rats against Lipopolysaccharide (LPS) -induced pyrexia. *P. longifolia* Benth. & Hook. f. var. *Pendula* (Annonaceae) is an evergreen tropical tree well known for its numerous medicinal properties. Methanol extracts of the leaves, stem bark and root of the plant were tested for their antipyretic activities at doses of 30, 100 and 300 mg kg^{-1} body weight using LPS-induced antipyretic activity model. All extracts showed significant ($p < 0.001$) dose-dependent antipyretic activity. At 300 mg kg^{-1}, all extracts exhibited activities higher than that of Acetylsalicyclic acid (Aspirin) whose percentage inhibition of pyrexia was 86%. The root extract was the most active with a percentage inhibition of 127.5%, followed by the leaf extract (123.0%) and the stem bark extract (99.2%). This study proves *P. longifolia* as an effective antipyretic agent and could be used as an adjunct in the treatment of other ailments.
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Keywords*: Polyalthia longifolia*, Lipopolysaccharide-induced fever, Antipyretic

INTRODUCTION

Pyrexia is caused in response to infection, tissue damage, inflammation and other diseased conditions. It is the body's natural way of creating an environment where infectious agents cannot survive (Shah and Seth, 2010). Prolonged or high fever however, often increases disease progression by increasing tissue catabolism, dehydration and other existing complaints, as in the case of patients suffering from the human immunodeficiency virus (Veugelers *et al.,* 1997).

Most antipyretic drugs function by inhibiting the expression of cyclooxygenase 2 (COX-2) to reduce the elevated body temperature by inhibiting the biosynthesis of prostaglandin E2 (PGE2) as reported by Shah and Seth, (2010). However, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of the brain and heart muscles (Elumalai *et al.,* 2012). Natural COX-2 inhibitors however have been reported to lower selectivity with fewer side effects (Cheng *et al.*, 2005). It is therefore essential to investigate traditionally used antipyretic agents of alternatives to these toxic synthetic antipyretic agents.

P. longifolia var. *pendula* (Annonaceae) is a single-stemmed tree which thrives in the tropics. Various parts of the plant have been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis (Katkar *et al.,* 2010). A number of biologically active compounds have been isolated from the leaves, stem and stem bark of the plant. Such include aporphine and azafluorene alkaloids (Wu *et al.,* 1990), proanthocyanidins (Nair and Chanda, 2006), clerodane-type diterpenoids (Matharanda-Murpy *et al.,* 2005) and sesquiterpene compounds

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(Ogunbimi *et al.,* 2007).These have been studied for various biological activities like anticancer (Chen *et al.,* 2003), antimicrobial (Faiz *et al.,* 2008), anti-inflammatory (Ramakrishna *et al.,* 2000), hypotensive (Saleem *et al*., 2005), antiulcer (Malairajan *et al.,* 2008), hypoglycaemic (Nair *et al.,* 2007) and antioxidant (Chang *et al.,* 2008). There is however, no scientific evidence supporting the traditional use of *P. longifolia* var. *pendula* as an effective remedy for fever.

The present study therefore aimed at evaluating the antipyretic activities of crude methanol extracts obtained from the root, stem bark and leaves of *P. longifolia* var. *pendula*, using the LPS-induced model in rats. This is to provide evidence for the potential role of *P. longifolia* var. *pendula* as a remedy for fever.

MATERIALS AND METHODS

Collection and preparation of plant materials

The leaves, stem bark and root of *P. longifolia* were collected in Kumasi in the Ashanti Region of Ghana and were authenticated by Mr. G. H. Sam at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. A voucher specimen (FP/ 097/11) of the plant has been deposited in the Department of Herbal Medicine, KNUST. The various parts were sun dried for two weeks and milled into coarse powder.

Extraction of plant material

Five hundred grams (500 g) of each plant part was weighed and sohxlet extracted in methanol for 8 hours. The liquid extracts were evaporated into a syrupy mass under reduced pressure using a rotary evaporator at 40^0C and vacuum-dried for 24 hours. The crude extracts were kept in a desiccator until required for use. The yields per gram of the extracts were 13.5%, 14.3% and 16.2% for the root, leaf and stem bark respectively.

Phytochemical screening

The presence of secondary metabolites in the powdered samples was investigated following simple qualitative methods by Trease and Evans, (2009). The plant samples were investigated for saponins, flavonoids, alkaloids, glycosides, phytosterols and terpenoids.

Animals

Sixty five (65) female albino rats (Wistar strain) weighing 140-180 g were used for the experiment. The animals were obtained from the Department of Pharmacology, KNUST. Rats were kept in metal cages and divided into 13 groups of 5 animals each. The animals were acclimatized to experimental conditions for 48 hours prior to the study and kept under standard conditions of room temperature (25ºC) and a 12 hour light/darkness cycle. They were starved overnight prior to the experiment while allowing access to drinking water.

Treatment doses

The crude extracts were emulsified in 2% Tween 80 and dissolved in normal saline at doses of 30, 100 and 300 mg kg^{-1} body weight. The same treatment doses were used for Acetylsalicylic acid (Sigma Aldrich Co., USA) which served as the positive control. Lipopolysaccharide of Gram negative *Escherichia coli* (Sigma Aldrich Co., USA) was prepared at a dose of 50 μg kg^{-1} body weight (Chomchuen *et al.,* 2010).

Antipyretic activity

The method of Santos and Rao, (1998) was modified and used for the assessment of the antipyretic activity of *P. longifolia*. Basal rectal temperature of each rat was measured using a digital thermometer inserted about 2 cm deep into the rectum. Fever was induced by intramuscular injection of 50 μg kg^{-1} dose of LPS in the right thigh of each animal. Rectal temperatures were measured and recorded 1 hour after injection. Doses (30, 100 and 300 mg kg^{-1} body weight) of the extracts were administered orally to the respective groups. Tween 80 in Normal saline was orally administered to the negative control group. Rectal temperatures were taken and recorded hourly for five hours. Changes in rectal temperature expressed as the difference from the basal value were determined.

Statistical analysis of data

The results were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's *multiple comparison* test for all the treated groups. Sigma Plot

11.0 was used for all statistical analyses. Values were expressed as Mean ± SEM. P values ≤ 0.001 were considered statistically significant.

RESULTS

Phytochemical screening

Results from the phytochemical analysis conducted indicated the presence of alkaloids and terpenoids in the powdered materials (Table 1).

Antipyretic activity

All the extracts generally exhibited significant ($p \leq 0001$) dose-dependent antipyretic activities. Animals groups that were administered with 300 mg $kg^{-1}$of the root and stem bark extracts had their temperatures falling below their initial temperatures (Figs. 1 and 2). Percentage inhibition of LPS-induced fever ranged between 53 and 127.4%. At the dose of 300 mg kg^{-1}, all the extracts exhibited activities greater than the standard drug, aspirin (Table 2). At 300 mg kg^{-1}, root extracts showed the highest inhibition of fever (Fig. 3).

Table 1: Phytochemical constituents from the leaves, stem bark and root of *P. longifolia*

Phytochemical constituent	Leaf	Stem bark	Root
Alkaloids	+	+	+
Tannins	-	-	-
Terpenoids	+	+	+
Flavonoids	-	-	-
Saponin Glycosides	-	-	+
Reducing sugars	-	+	+

Table 2: Percentage inhibition of LPS-induced pyrexia of *P. longifolia* at different doses

EXTRACT	Dose (mg kg^{-1})	% Inhibition (%)
P. longifolia leaf extract (PLE_{LV})	30	98.1%
	100	97.3%
	300	123.0%
P. longifolia stem bark extract (PLE_{ST})	30	53.0%
	100	93.2%
	300	99.2%
P. longifolia root extract (PLE_{RT})	30	82.8%
	100	78.3%
	300	127.4%
Aspirin	30	26.7%
	100	102.1%
	300	86.1%

DISCUSSION

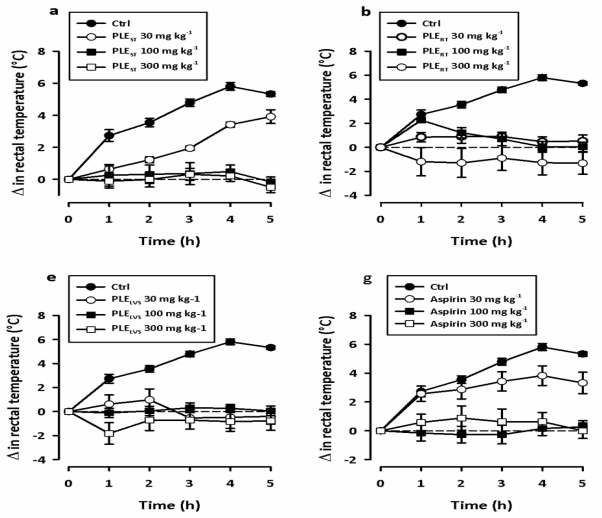

Figure 1: Change in rectal temperature (^{0}C) against time (h) graphs of the stem bark (a), root (b), leaves (e) and aspirin (g)

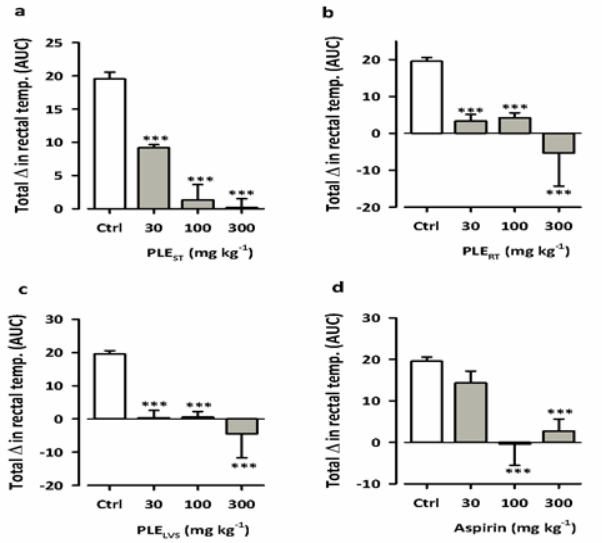

Figure 2: Total change rectal temperature expressed as area under the curve of the stem bark (a), root (b), leaves (c), and aspirin (d)

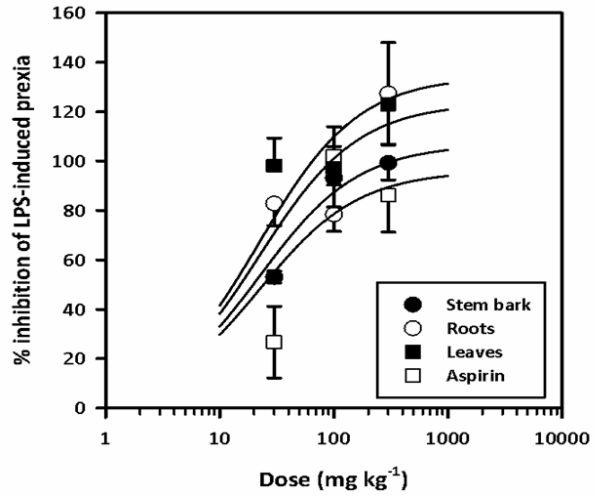

Figure 3: Percentage inhibition of LPS-induced pyrexia against dose concentration (mg/kg)

The present study was performed to determine the antipyretic activities of the leaves, stem bark and roots of *Polyalthia longifolia* var. *pendula* using the LPS-induced fever model in rats. Results from the phytochemical analysis conducted indicated the presence of alkaloids and terpenoids in all the three plant parts. From previous studies, other plants containing such constituents have been associated with antipyretic activities.

Deepa *et al.,* (2009) investigated the antipyretic activity of *Vernonia cinerea* which was known to contain alkaloids, saponins and terpenoids to be its major constituents. *Androphragis paniculata* and *Adhatoda vasica*, reported to contain andrographolide, a diterpene and vasicine, an alkaloid respectively have also been reported to possess antipyretic activities against yeast-induced fever in rats (Chandra *et al.,* 2010). Rosmarinic acid from *Rosmarinus officinalis* has also been reported to inhibit prostaglandin synthesis (Shah and Seth, 2010).

The antipyretics in common use today include acetaminophen, aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDS). The principal action of antipyretics rest in their ability to inhibit the enzyme cyclooxygenase (COX) and interrupt the synthesis of inflammatory prostaglandins (Jongchanapong *et al.,* 2010). They also suppress the production of pyrogenic cytokines such as TNF -α and IL-β (Aronoff and Nelson, 2001).

P. longifolia var. *Pendula* may therefore follow a similar mechanism of action to exhibit such high activity. The presence of the phytoconstituents investigated may partly be attributed to such high activities.

CONCLUSION

The leaves, stem bark and root extracts of *P. longifolia* var. *Pendula* possess high antipyretic activities comparable to aspirin. This may provide in part, scientific evidence for its use as a traditional remedy for fever. Further bioactivity guided fractionation of the extracts to determine specific compounds responsible for such high activities is ongoing our laboratories.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Prevalence and determinants of proteinuria among type 2 diabetics in Kumasi, Ghana

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Diabetic nephropathy is the leading cause of end stage kidney disease among type 2 diabetics worldwide. Proteinuria has been noted to be the cardinal symptom of progressive loss of renal function. This study examined the impact of duration of diabetes, demography (age, gender) and metabolic factors on the frequency of proteinuria among type 2 diabetics visiting the Komfo Anokye Teaching Hospital (KATH). In this cross-sectional study, 350 type 2 diabetics aged between 28-87 years were randomly selected from January to April 2004, and parameters estimated include fasting blood glucose (FBS), body mass index (BMI), urine protein and blood pressure. Proteinuria among the study cohorts was graded no proteinuria, mild proteinuria to heavy proteinuria. The frequency of proteinuria for the varied grades in type 2 diabetics enrolled in the study ranged from 73.3% (no proteinuria), 15.2% (mild proteinuria) and 15.6% (heavy proteinuria). 1(100%) patient with heavy proteinuria presented with grade 3 hypertension; and 4(33.3%) and 11(20.8%) patients presented with grade 1 and isolated systolic hypertension respectively. Multiple logistic regression analysis showed study participants with duration of diabetes ranging from 11-15 years (OR=2.8; 95% CI=1.1-7.2; p=0.028) and 16-20 years (OR=5.6; 95% CI=1.4-22.5; p=0.016) were at an increased risk of proteinuria. The frequency of nephropathy is promoted independently by advanced age, hypertension and duration of diabetes.

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Keywords*:* Proteinuria, type 2 diabetes, obesity, hypertension

INTRODUCTION

Diabetes mellitus is a clinical syndrome associated with insulin deficiency, inefficiency or both (Harris and Zimmet, 1997). The world health organization (WHO) estimates that over 170 million people worldwide are presenting with diabetes with the number possibly rising to 370 million in the next 20 years (USRD, 2004). One of the complications of type 2 diabetes mellitus (T2DM) is nephropathy characterized by increased excretion of protein in the urine which is presently the leading attributable cause of chronic kidney disease (CKD) (USRD, 2004).

To date, there is a paucity of information on proteinuria (macroalbuminuria) among type 2 diabetics in Ghana; however, studies conducted in Nigeria and other African countries have reported highly varying prevalence rates ranging from as low as 7.0% to 82.5% (Lutale *et al.*, 2007; Balogun and Abbiyesuku, 2011).

Several studies have concluded that factors such as age, gender, diet and obesity, hypertension and proteinuria influence the progression of diabetic nephropathy (Rossing *et al.*, 2004; Leehey *et al.*, 2005; Imai *et al.*, 2008). Subsequently, glycaemic

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control and the reno-protective effect of angiotensin converting enzyme inhibitors and blockers on proteinuria have been identified as factors that influence proteinuria in T2DM (Klahr *et al.*, 1994; Peterson *et al.*, 1995; Mandal and Hiebert, 2008). The interplay of these factors in the development and progression of proteinuria and subsequent diabetes associated nephropathy among Ghanaian diabetics has not been well elucidated. This study therefore examined the impact of age, obesity, hypertension, blood glucose concentration and duration of diabetes on the frequency of proteinuria among T2DM patients.

MATERIALS AND METHODS

Study area and subjects

This randomised cross-sectional study was conducted at the Diabetic clinic of the Komfo Anokye Teaching Hospital (KATH), Kumasi, in the Ashanti region of Ghana from January to April 2004. A total of 350 subjects comprising 251 females and 99 males with ages ranging from 28-87 years were selected from the diabetic centre of the KATH. A self-structured questionnaire was administered to each participant after recruitment into the study. The participation of the respondents who were all indigenes of Ghana was voluntary and informed consent was obtained from each study participants prior to enrolment into the study. The study was approved by the School of Medical Sciences and KATH Committee on Human Research, Publication and Ethics (SMS/KATH/CHRPE).

Inclusion criteria

All type 2 diabetics with ages ranging from 28 years and who were on hypoglycaemic agents or on diet therapy, but not on insulin therapy were enrolled into the study. Confirmation of diagnosis was made from patient folders before being recruited into the study.

Exclusion criteria

Type 1 diabetics and type 2 diabetics undergoing any form of dialysis were excluded from the study. Diabetics on insulin therapy were excluded from the study.

Blood Sample collection

Two millilitres (2 ml) of venous blood was drawn from each study participant after an overnight fast (12-14 hours) and dispensed into fluoride oxalate tubes. After centrifugation at 1500 *g* for 3 minutes, the plasma was aliquoted into cryovials and stored at - 80°C until assayed.

Fasting blood sugar

This was estimated using the glucose oxidase/peroxidase method (Trinder, 1969) and the colour developed was measured with a spectrophotometer [(Spectronic-20), 820 Linden Avenue, Rochester, NY 14625, USA] at a wavelength of 500 nm.

Urine collection and estimation of urine protein

Early morning urine samples collected into clean, wide mouth and leak proof containers were obtained from the participants and preserved with boric acid (0.1 g) for every 10 ml of urine. Proteinuria (semi-quantitative) was assessed using dipstick (CYBOW™ DFI Co Ltd, Gimhae-City, Republic of Korea) and confirmed with the sulphosalicylic acid method. Proteinuria was defined as none when the dipstick test turns out negative; mild for dipstick results ranging from trace to 1+ and heavy for dipstick results ranging from 2+ to 4+.

Anthropometric variables

Height to the nearest centimetre without shoes was measured with a wall-mounted ruler and weight to the nearest 0.1 kg in light clothing was measured using a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China). Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2).

Blood Pressure (using Krotkoff 1 and 5)

Blood pressure was measured by trained personnel using a mercury sphygmomanometer and a stethoscope. All measurements were in accordance with recommendations of the American Heart Association (Kirkendall *et al.*, 1967). Mean values of duplicate measurements were recorded as the blood pressure. Hypertension was graded as normal when the systolic blood pressure (SBP) is less than 120 mm Hg and diastolic blood pressure (DBP) is less than

80 mm Hg; pre-hypertension: SBP= 120-139 or *DBP* =80-89; Stage 1 hypertension: SBP=140-159 *or* DBP=90-99; Stage 2 hypertension: SBP >160 *or* *DBP*>100 (Chobanian *et al.*, 2003).

Calculation of Mean Arterial Pressure (MAP)

The mean arterial pressure was estimated using the formula:

$$MAP \cong DP + \frac{1}{3}(SP - DP)$$

Statistical analysis

Results are expressed as means ± SD. Unpaired *t*-test was used to compare mean values of continuous variables and χ^2test statistic was used to compare all categorical variables. For all statistical comparisons, a p-value<0.05 was considered as statistically significant. Multivariate logistic regression was used to estimate the odds ratios of risk factors of proteinuria after adjusting for age and sex. GraphPad Prism version 5.00 for windows (GraphPad software, San Diego California USA, www.graphpad.com) and SYSTAT version 12 (SYSTAT software, 239 Western Street, Suite F Fairfield, CA, USA, www.systat.com) were used for all statistical analysis.

RESULTS

Clinical characteristics of the study population

The mean duration of diabetes was significantly higher in the group with heavy proteinuria compared to the groups with mild and no proteinuria respectively. The mean age, diastolic blood pressure, and fasting glucose of the group with heavy proteinuria are significantly higher compared to the group without proteinuria. The mean arterial pressure and systolic blood pressure of the heavy proteinuria group were significantly higher compared to the mild proteinuria group (Table 1).

Frequency of proteinuria in relation to body mass index

The prevalence of proteinuria among the study respondents stratified by body mass index are as shown in Table 2. Out of the total of 16 (4.7%) underweight study participants, 62.5% (10/245) had no proteinuria, 25% (4/49) had mild proteinuria and 12.5% (2/47) had heavy proteinuria. Out of the 137 (40.2) study participants with normal weight, 73.7% (101/245) had no proteinuria, 15.3% (21/49) had mild proteinuria and 10.9% (15/47) had heavy proteinuria. For the 138 (40.0%) pre-obese study participants, 104 (75.4%) had no proteinuria, 14 (10.1%) had mild proteinuria and 20 (14.5%) had heavy proteinuria. For the 50 (14.7%) obese study participants, 60.0% (30/245) had no proteinuria with 20.0% each having mild (10/49) and heavy (10/47) proteinuria respectively.

Table 1: Demographic, clinical and biochemical characteristics of the study participants stratified by the level of proteinuria on dipstick

Variable	Total (n = 341)	None (n = 245)	Mild (n = 49)	Heavy (n = 47)
Duration of diabetes (years)	5.8 ± 4.8	5.2 ± 4.1	6.0 ± 5.7	8.5 ± 5.8*†
Age (years)	54.9 ± 11.0	53.9 ± 11.1	56.4 ± 8.7	58.9 ± 11.8*
MAP	94.6 ± 10.2	92.9 ± 7.7	97.8 ± 15.4*	100.3 ± 12.1*
SBP (mm Hg)	130.3 ± 14.9	127.7 ± 13.0	134.6 ± 14.6*	139.3 ± 19.5*
DBP (mm Hg)	76.4 ± 7.0	75.5 ± 6.1	77.2 ± 7.5	80.3 ± 9.1*
BMI (kg m^{-2})	25.5 ± 4.4	25.4 ± 4.1	25.0 ± 5.5	26.6 ± 4.8
Fasting blood glucose (mmol L^{-1})	9.2 ± 3.0	9.0 ± 2.8	9.4 ± 3.4	10.3 ± 3.3*

p<0.05; **p<0.01; ***p<0.001; *significantly different from group with no proteinuria; †significantly different from group with mild proteinuria; MAP = mean arterial pressure; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; None = negative; Mild = trace to 1+; Heavy = 2+ to 4+

Table 2: Prevalence of proteinuria stratified by body mass index and the level of proteinuria on dipstick

Body Mass Index	Total (n = 341)	None (n = 245)	Mild (n = 49)	Heavy (n = 47)
Underweight (<18.5)	16(4.7)	10(62.5)	4(25.0)	2(12.5)
Normal (18.5-24.9)	137(40.2)	101(73.7)	21(15.3)	15(10.9)
Pre-obese (25.0-29.9)	138(40.0)	104(75.4)	14(10.1)	20(14.5)
Obese (≥30.0)	50(14.7)	30(60.0)	10(20.0)	10(20.0)

Prevalence of proteinuria in relation to fasting blood glucose

Analyses of the frequency of proteinuria in relation to plasma fasting blood glucose concentration are as shown in Table 3. For the 43 (12.6%) study participants with normal blood glucose concentration, 74.4% (32/245) had no proteinuria, 11.6% (5/49) had mild proteinuria and 14.0% (6/47) had heavy proteinuria. For the 48 (14.1%) study participants with impaired fasting glucose, 83.3% (40/245) had no proteinuria, 12.5% (6/49) had mild proteinuria and 4.2% (2/47) had heavy proteinuria. For the 250 (73.3%) study participants with fasting blood glucose greater 7.0 mmol L^{-1}, 69.2% (173/245) had no proteinuria, 15.2% (38/49) had mild proteinuria and 15.6% (39/47) had heavy proteinuria (Table 3).

Duration of diabetes and frequency of proteinuria

Figure 1 assesses the frequency of proteinuria among the study participants in relation to the duration of diabetes. The frequency of heavy proteinuria showed a gradual increase from a frequency of 7.8% in study participants with diabetes duration of <1 year, through 8.1% (in the 2 – 5 years diabetes duration group), 14.5% (in the 6 – 10 years diabetes duration group), 33.3% (in the 11 – 15 years diabetes duration group) and 60.0% in the 16 – 20 diabetes duration group. None of the study participants with >20 years diabetes duration showed visible signs of heavy proteinuria. Mild proteinuria, from a frequency of 14.1% in participants with <1 year diabetes duration rose to 14.5% in the 2 – 5 and 6 – 10 years duration of diabetes groups respectively before gradually declining to 0.0% in the 16 – 20 and >20 year duration of diabetes respectively. Generally, the frequency of no proteinuria in the study participants decreased gradually from 78.1% in the ≤1 year duration of diabetes group through 77.4% (2 – 5 years), 70.0% (6 – 10 years), 56.7% (11 – 15 years), 40.0% (16 – 20 years) and 33.3% (>20 years).

Blood pressure categories and frequency of proteinuria

From Table 4, with the exception of 6 (8.2%) study participants with optimal blood pressure who tested positive for heavy proteinuria, none of the study participants with normal blood pressure and prehypertension tested positive for heavy proteinuria.

Table 3: Frequency of proteinuria in relation to the level fasting blood glucose as well as the level of proteinuria on dipstick

Fasting blood glucose	Total (n = 341)	None (n = 245)	Mild (n = 49)	Heavy (n = 47)
Normal (<6.1)	43(12.6)	32(74.4)	5(11.6)	6(14.0)
IFG (≥6.1 – <7.0)	48(14.1)	40(83.3)	6(12.5)	2(4.2)
DM (>7.0)	250(73.3)	173(69.2)	38(15.2)	39(15.6)

IFG = impaired fasting glucose; DM = diabetes mellitus

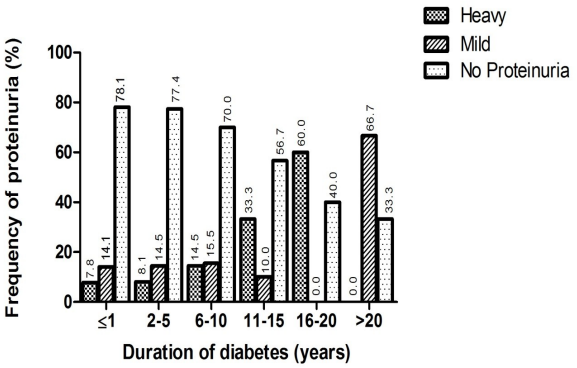

Figure 1: Relationship between frequency of proteinuria and duration of diabetes.

There was a gradual increase in the frequency of participants with mild proteinuria from 5.5% in participants with optimal blood pressure through 12.5% for participants with normal blood pressure and 37.5% for participants with pre-hypertension. For the blood pressure categories, 86.3% of the participants with optimal blood pressure had no proteinuria; 87.5% of the participants with normal blood pressure had no proteinuria and 62.5% of the participants with pre-hypertension had no proteinuria. On grading hypertension, 33.3% of the participants with grade 1 hypertension had heavy proteinuria, 16.7% had mild proteinuria and 50.0% had no proteinuria. The study participant with grade 3 hypertension had heavy proteinuria. Among the study participants with isolated systolic hypertension, 20.8% had heavy proteinuria, 22.6% had mild proteinuria and 56.6% had no proteinuria.

Table 4: Frequency of proteinuria in relation to blood pressure categories as well as the level of proteinuria on dipstick

Blood Pressure Category	Systolic	Diastolic	Total (n= 341)	None	Mild	Heavy
Optimal	<120	<80	73(21.4)	63(86.3)	4(5.5)	6(8.2)
Normal	120 – 129	80 – 84	8(2.3)	7(87.5)	1(12.5)	0(0.0)
Prehypertension	130 – 139	85 – 89	8(2.3)	5(62.5)	3(37.5)	0(0.0)
Hypertension						
Grade 1	140 – 159	90 – 99	12(3.5)	6(50.0)	2(16.7)	4(33.3)
Grade 2	160 – 179	100 – 109	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Grade 3	≥180	>110	1(0.3)	0(0.0)	0(0.0)	1(100.0)
Isolated systolic	≥140	<90	53(15.5)	30(56.6)	12(22.6)	11(20.8)

Odds analysis of some selected variables and their association with proteinuria

Multivariate logistic regression analysis of selected variables and their association with proteinuria are as shown in Table 5. Sex was not significantly associated with proteinuria. Testing positive for urine glucose was associated with approximately 2 times risk of testing positive for proteinuria (OR = 1.9; p = 0.023). On the analysis of BP, systolic (≥140 mm Hg) and diastolic (≥90 mm Hg) blood pressure were both linked with 3 times risk for proteinuria (p = 0.000 and p = 0.019 respectively). Analysis of age categories for the participants showed a marginal risk for proteinuria within the 50 – 60 year age group (OR = 4.4; p = 0.051) and the 72 – 82 year age group (OR = 5.5; p = 0.053) respectively. The 61 – 71 year age group however showed a significant association with proteinuria with a 7.2 times risk (p = 0.010). The fasting blood glucose concentration of the participants was not significantly associated with proteinuria. Stratification of the study participants by BMI showed no significant association between the classes of BMI and proteinuria (p >0.05). On analysing diabetes duration however, periods from 11 – 15 years and 16 – 20 years were linked with a significant presence of proteinuria with odds ratios of 2.8 and 5.6 respectively.

Table 5: Multivariate logistic regression of factors associated with proteinuria

Variables	OR(95% CI)	P value	SE
Gender			
Female*	1		
Male	1.2(0.7 - 1.9)	0.597	0.304
MAP	1.1(1.0 - 1.1)	0.000	0.015
Urine glucose			
Positive	1.9(1.1 - 3.2)	0.023	0.506
BP (mm Hg)			
Systolic (≥140)	3.0(1.8 - 5.2)	0.000	0.833
Diastolic (≥90)	3.0(1.2 - 7.8)	0.019	1.453
Age (years)			
28 – 38*	1		
39 – 49	3.8(0.8 - 17.6)	0.089	2.971
50 – 60	4.4(1.0 -19.6)	0.051	3.356
61 – 71	7.2(1.6 - 32.7)	0.010	5.574
72 – 82	5.5(1.0 - 31.5)	0.053	4.908
≥83	–	–	–
Fasting Glucose (mmol L^{-1})			
<6.1 *	1		
6.1 – 7.0	1.0(0.3 - 3.4)	0.976	0.623
>7.0	1.8(0.6 - 5.5)	0.330	1.024
BMI (kg m^{-2})			
Underweight*	1		
Normal	0.7(0.2 - 2.2)	0.572	0.418
Pre-obese	0.7(0.2 - 2.1)	0.486	0.388
Obese	1.3(0.4 - 4.5)	0.642	0.826
Diabetes duration (years)			
≤1*	1		
2 – 5	1.1(0.5 - 2.2)	0.839	0.401
6 – 10	1.6(0.8 - 3.3)	0.186	0.590
11 – 15	2.8(1.1 - 7.2)	0.028	1.351
16 – 20	5.6(1.4 - 22.5)	0.016	3.968
>20	7.4(0.6 - 88.0)	0.112	9.369

**** Reference variables; OR = odds ratio; CI = confidence interval; SE = standard error of the odds ratio estimates; MAP =mean arterial pressure; BP = blood pressure***

DISCUSSION

This study evaluated the effects of blood pressure, obesity, fasting blood glucose concentration, duration of diabetes and age on the frequency of proteinuria among type 2 diabetics. The degree of proteinuria increased with advancing age, blood pressure and the duration of the diabetes. Such significant association with proteinuria (appearance of protein in urine) therefore presents each of the assessed variables as independent risk factors to the development of nephropathy (kidney damage).

The observed association between heavy proteinuria and the mean duration of diabetes among study participants from this study corroborates similar observations made in numerous studies and confirms duration of diabetes as an important factor in the development of proteinuria (Klein *et al.*, 1995; Stratton *et al.*, 2000).

Several studies have identified male gender and older age of onset of diabetes as independent risk factors for proteinuria in T2DM (Ballard *et al.*, 1988; Gall *et al.*, 1991; Klein *et al.*, 1995). From this study, sex and for that matter being a male was not a significant risk factor for proteinuria as observed from the multivariate logistic regression analysis. However, older age of onset, specifically age ≥50 years was significantly associated with proteinuria. It therefore suffices to infer from the results that irrespective of sex, age of onset of T2DM greatly modifies the presence or absence of proteinuria.

Elevated blood pressure, be it systolic or diastolic was significantly associated with proteinuria and in previous studies has been found to be an independent risk factor for the development and progression of proteinuria in T2DM (Rossing *et al.*, 2004). Hypertensive nephrosclerosis has been identified as the foremost cause of end stage kidney disease (USRD, 1999). Consequently, treatment with antihypertensives has been shown to reduce the risk of developing proteinuria and also slowing down the progression of renal injury once nephropathy develops (Parving, 1998). A further stratification of the study participants by grade of hypertension showed increases in mild and heavy proteinuria as hyperten-

sive grade increased. The anti-hypertensive proteinuria risk reduction could however not be assessed in this study cohort as per the design of the study, information on anti-hypertensive treatment was not sought. The finding of 15.5% of the study participants with ISH is consistent with previously published works (Trevisan *et al.*, 2002; Bakris, 2004) and could be due to the fact that type 2 diabetic patients are mostly burdened with ISH.

The estimated proteinuria prevalence among the obese participants reported in this study is far lower than that reported from the work of Alwakeel *et al.,* (2011). The observed difference could be attributed to the methodology adapted for proteinuria estimation. In this study, proteinuria was assessed using early morning urine whilst in the study of Alwakeel *et al.,* (2011) proteinuria was assessed utilizing 24 hour urine.

The high rate of obesity with its associated high proteinuria (mild and heavy) observed in this study is in conformity with the findings of (Praga and Morales, 2006). Weight loss, thus, has been identified as an inducer of a reduction in proteinuria among patients with varied causes of proteinuria for which weight gain is an attributable risk factor.

Obesity, hypertension and duration of diabetes independently contribute to the development of proteinuria in T2DM. Elevated BMI has been recognized as a risk factor for increased proteinuria among diabetics in several studies (Anastasio *et al.*, 2000; Ramirez *et al.*, 2002) however no significant association was observed in the classes of BMI and proteinuria from the multivariate analysis conducted in this study. BMI has been touted as being an insensitive method for assessing adiposity and this fact coupled with dietary therapy and restriction in this cohort of study participants could account for the lack of an observed significant difference in the BMI classes and proteinuria. The effect of hyperglycaemia on proteinuria is well documented (Stratton *et al.*, 2000). Fasting glucose concentration was not significantly associated with proteinuria from the multivariate analysis in this study but duration of diabetes (11 – 20 years) was significantly associated with proteinuria. The 15% frequency of heavy proteinuria found in participants with elevated FBS levels in this study could thus be attributed to good glycaemic control practices among this cohort of participants' couples with a reduced duration of diabetes which could be the sole deciding factor. A number of studies (Ramirez *et al.*, 2002; Al-Homrany and Abdelmoneim, 2004) have observed using logistic regression models that those with high SBP and DBP besides longer duration of diabetes have higher levels of proteinuria. This finding is corroborated by results from this study. From the multivariate analysis, the above named factors individually contribute to kidney damage through glomerulosclerosis ultimately resulting in hyperfiltration and consequently proteinuria, which is a cardinal sign of overt nephropathy.

CONCLUSION

The frequency of proteinuria in Ghanaians with T2DM was 13.8%. Significant predictors of proteinuria included age, duration of diabetes, hypertension and duration of diabetes. Strategies to mitigate the occurrence of nephropathy should be targeted at glycaemic and hypertension control.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Proficiency testing of total serum cholesterol assay by the ATAC 8000® random access chemistry auto analyzer at the Komfo Anokye teaching hospital

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Cardiovascular disease has become a leading cause of death with hypercholestrolaemia being one of the most important and implicated modifiable risk factor in both developed and developing countries. Analysis of cholesterol is thus important and many analytical techniques have been developed. Different methods for determining total cholesterol can produce varying laboratory results, thus illustrating the importance of quality control. The present study, therefore, aims at comparing the ATAC 8000 Random Access Chemistry Autoanalyzer used at the KATH, the manual enzymatic method and the WHO recommended manual method with reference to total cholesterol assay. A 20 day replication run was conducted utilizing stabilized human control serum. Methods comparison was then performed on 90 patient samples using the ATAC 8000 autoanalyzer adopted method for estimating cholesterol (enzymatic end point method) as the test method, the manual enzymatic end point method and the WHO recommended Liebermann-Burchard method as comparative methods, to analyse total serum cholesterol. ATAC 8000 gave the lowest CV of 3.0% and total error (TE) of 6.5%, followed by the Liebermann-Burchard method (CV of 4.6%; TE of 9.0%) and the manual enzymatic method (CV of 7.0%; TE of 13.7%). The recommended CV ranges from 3-5% with TE being ≤ 8.9%. The autoanalyzer consistently generated results that were higher than the other methods with good precision and accuracy. Based on the capability index (Cp), ATAC 8000 had the highest Cp of 2.5 followed by Liebermann-Burchard method with a Cp of 1.8. The higher the Cp, the lower the risk of jumping the tolerance limits and therefore the higher the quality. The Bland-Altman analysis showed good agreement between the ATAC 8000 and other methods. Total analytical error, capability index and CV produced by the method adapted for cholesterol estimation by ATAC 8000 Random Access Chemistry autoanalyzer is acceptable since all are within the recommended and set ranges for total cholesterol.
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Keywords*:* ATAC 8000, Liebermann-Burchard Method, Enzymatic endpoint method, Total Cholesterol

INTRODUCTION

Cardiovascular disease (CVD) is said to be a major cause of morbidity and mortality worldwide (Murray and Lopez, 1997). Even though mortality associated with CVD has declined in economically developed countries, the epidemic of CVD in recent times has been observed in developing countries (Reddy and Yusuf, 1998). This observed trend has resulted, in a large part, from the economic growth and associated socio-demographic changes that have occurred over recent decades. Notwithstanding the declines in illnesses from infectious diseases, changes in lifestyle and diet have led to increased burden of CVD and other chronic diseases with an overall resultant fall in life expectancy (He *et al.*, 2004; Reddy and Yusuf, 1998).

With high blood cholesterol being one of the most

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important modifiable risk factor for CVD and its associated mortality (He *et al.*, 2004; LaRosa *et al.*, 1999), there is, however, paucity of data on the population levels of serum cholesterol in developing countries (He *et al.*, 2004; Hughes *et al.*, 1997). There are indications of people being quite well aware of their blood pressure levels and possible hypertension in many countries. The same can however not be said for cholesterol levels and general awareness of hypercholesterolaemia. The prevalence of hypercholesterolaemia varies considerably between countries (Cutter *et al.*, 2001; Tolonen *et al.*, 2005; Ulmer *et al.*, 2001), within countries, between different areas and population groups as well as the method of estimation employed (Cirera *et al.*, 1998; Polednak, 1992; Ulmer *et al.*, 2001).

Several methods for the estimation of serum cholesterol have been outlined for which some are still being evaluated for their precision, accuracy and recovery. Differing methods of determining total cholesterol can produce varying laboratory results, thus illustrating the importance of quality control in the measurement of total cholesterol. Quality control plays a vital role in the harmonization of results for total cholesterol. Accurate analysis of cholesterol is therefore imperative given the fact that high serum cholesterol is a well-noted health hazard (Thompson and Wood, 1995).

This study therefore aims at comparing the ATAC 8000 Random Access Chemistry autoanalyzer used at the Clinical Chemistry Department of the Komfo Anokye Teaching Hospital (KATH), the manual enzymatic method, and the WHO recommended manual method in the assay of total serum cholesterol. KATH is a referral hospital for the Ashanti Region as well as for the Northern sector of the country. In separate studies, (Owiredu *et al.*, 2007a; Owiredu *et al.*, 2007b) have reported unreliability in comparative studies on electrolytes and results of liver function test turned out by the ATAC 8000 Random Access Chemistry Autoanalyzer.

MATERIALS AND METHODS

Study period and site

The study was conducted at the Clinical Chemistry Department of KATH spanning from March 2008 to April, 2008.

Subjects and Specimen Collection

Without the use of a tourniquet, venous blood samples were collected into Vacutainer® plain tubes after an overnight fast (12 – 16 hours) from ninety (90) adult patients visiting the KATH Clinical Chemistry Laboratory for lipid profile test after informed consent. The blood was allowed to clot, centrifuged at 500 g for 15 minutes within 30 minutes of sample collection and the serum stored at -80 °C until assayed. Samples which were haemolysed or showed signs of haemolysis were excluded from the study. Analysis of total serum cholesterol was done for the 90 patient samples using the ATAC 8000 Random Access Chemistry autoanalyzer (end-point assay type), the manual enzymatic end-point method and the WHO recommended manual method (Liebermann-Burchard method).

Pooled human serum stabilized with 15% ethylene glycol was used in the replication study in order to obtain unbiased observations concerning the day-to-day performance of the assay. The stabilized human sera were prepared as recommended by the WHO (WHO-LAB/86.4). Residual serum samples from patients visiting the clinical laboratory were pooled in a 120 ml portion and stored at 4°C until assayed. Sera with apparent turbidity, excessive bilirubin, or haemolysis were excluded from the pool. After pooling, the sera were centrifuged in 15-mL volumes at 3000 g for 30 minutes, after which the chylomicrons at the meniscus were removed by manual aspiration. Before distribution, the subpools were verified to be free of human immunodeficiency virus (HIV) and hepatitis, although necessary precautionary measures were implemented during the entire process.

Standard Concentration

The study was specifically designed to have a short turnaround time so that the laboratory could be monitored closely. A reference curve was constructed from known total serum cholesterol standard for kit calibration and linearity determination of

the spectrophotometer used and the comparative analysis of the manual method and the autoanalyzer (ATAC 8000). The results of total serum cholesterol using the manual method was used as the 'target' values i.e. the best result the reference laboratory could obtain for the analyte within that sample.

Principle for the determination of total serum cholesterol (ATAC 8000 and the Manual Enzymatic Method)

Cholesterol esters are converted into their fatty acid and cholesterol components by cholesterol esterase. The cholesterol produced is converted to cholest-4-en-3-one and hydrogen peroxide by cholesterol oxidase in the presence of oxygen. The peroxide reacts with hydroxybenzoic acid (HBA) and 4-aminoantipyrine by the action of peroxidase to form the red colour-producing quinoneimine. The intensity of the red color produced is directly proportional to the total cholesterol in the sample when read at 500 nm.

WHO recommended Liebermann-Burchard Method

Cholesterol reacts with a strong acid medium of the combined reagent containing sulphosalicylic acid-glacial acetic acid, acetic anhydride and concentrated sulphuric acid to form a blue-green chromophore whose absorbance is measured at 600 nm.

Statistical Analysis

The results were presented as means. Methods comparison was done using Bland-Altman plot. Bland-Altman analysis estimates the difference in measurement values obtained by two methods on the same subject (Altman and Bland, 1986). The mean of such differences in a sample of subjects is the estimated bias (difference between methods) and the standard deviation (SD) of the differences measures random fluctuations around this mean. If the "limits of agreement" (mean difference ± 2SD) between two methods are not clinically important, one can use the two methods interchangeably. All statistical analyses and methods comparison were done using GraphPad Prism version 5.00 for windows (GraphPad software, San Diego California USA, www.graphpad.com) and MultiQC version 5.3.2.2 (www.multiqc.com).

RESULTS

Using the 20-day replication run on the pooled human serum, the obtained CVs for the various methods are as shown in Table 1. The manual enzymatic method could not meet the WHO recommended allowable CV of 3-5% for total cholesterol. The CV gives an indication of the precision of the method with high CV indicating greater degree of imprecision. The ATAC 8000 autoanalyzer gave the highest capability index (Cp) of 2.5, followed by that of the WHO recommended Liebermann-Burchard manual method (Cp = 1.8) and the manual enzymatic method (Cp = 1.2).

Table 1: Parameter estimates from repeatability studies for the three total cholesterol estimating methods

Parameters	ATAC 8000	Enzymatic	Liebermann-Burchard
Range (mmol L^{-1})	4.4–4.8	3.6–4.4	3.5–3.9
Mean (mmol L^{-1})	4.55	4.00	3.73
SD (mmol L^{-1})	0.15	0.28	0.17
CV (%)	3.30	7.00	4.60
Cp	2.50	1.20	1.80

SD – standard deviation; CV – coefficient of variation; Cp – capability index

The exponentially weighted moving average (EWMA) was calculated daily to detect the presence of process shift or bias. The EMWA is a cumulative score that weighs the earlier observations slightly lower than the subsequent observations in such a way as to automatically phase out distant observations almost entirely. The EWMA deviations at the end of the 20-day replication study was 0.3%, 3.8% and -2.7% for ATAC 8000 autoanalyzer, the manual enzymatic kit and the Liebermann-Burchard methods respectively. The manual enzymatic kit method yielded the highest EMWA of the three methods.

The total errors calculated were 6.5%, 13.7% and 9.0% for the ATAC 8000, the manual enzymatic and the Liebermann Burchard methods respectively. The performance index (Pp) for the ATAC 8000, the manual enzymatic method and the WHO reference method using control serum for 20 replicate runs yielded 1.1, 0.7 and 1.1 respectively as shown in Table 2.

Table 2: Performance studies for the various analytical methods used in estimating total cholesterol

Parameters	ATAC 8000 (N = 20)	Enzymatic (N = 20)	Liebermann-Burchard (N = 20)
TE	6.5	13.7	9
Pp	1.1	0.7	1.1

TE – total error [% bias + 1.96 (CV)]; Pp – performance index;

Comparison of the ATAC 8000 autoanalyzer and the manual enzymatic method with the WHO reference method was conducted using 90 patient samples by the Bland–Altman Plot. ATAC 8000 autoanalyzer and manual enzymatic turn out mean values that are about the same as compared to the WHO recommended Liebermann-Burchard manual methods. The general characteristics of the three methods are as shown in Tables 3 and 4.

Table 3. Parameter estimates for total cholesterol as determined with the three comparative methods for 90 patient samples

Parameters	Liebermann-Burchard (N = 90)	Enzymatic (N = 90)	ATAC 8000 (N = 90)
Mean	4.75	5.94	6.03
SD	2.01	2.16	1.36
Minimum	1.3	2.1	2.9
Maximum	12.4	12.1	9.2

SD – standard deviation

Table 4: Parameter estimates for Bland-Altman comparisons between the three test methods

Parameters	ATAC vs. ENZ	ATAC vs. LIEB	ENZ vs. LIEB
Bias	0.08	1.27	1.19
SD	1.66	2.31	2.46
95% limits of agreement	-3.17 – 3.34	-3.25 – 5.80	-3.64 – 6.02

ATAC = ATAC 8000 autoanalyzer; ENZ = enzymatic manual method; LIEB = WHO recommended manual methods (Liebermann-Burchard methods).

The Bland-Altman results for bias and agreement between the enzymatic manual method and the ATAC autoanalyzer method indicated the best agreement between the two methods for total cholesterol estimation followed by the enzymatic manual method and Liebermann-Burchard method and then the ATAC autoanalyzer and Liebermann-Burchard method as indicated in Table 4 and Figures 1A, 1B and 1C respectively.

DISCUSSION

This study aimed at quantifying total serum cholesterol using the ATAC 8000 autoanalyzer, the manual enzymatic kit method and the Liebermann-Burchard method with the aim of determining the quality and comparability of results generated by each of the methods. In assessing the quality of the determination of total serum cholesterol, factors of major consideration are imprecision, which has to be low and accuracy.

Accuracy, represents the reliability in performance evaluation of methods and is most frequently hampered by the fact that the control sera used in such evaluations are not comparable with the fresh human serum matrix (Thompson and Wood, 1995). Such existing variations thereby induce serious method, reagent, and analyzer-dependent differences that obscure assessment of the real laborato-

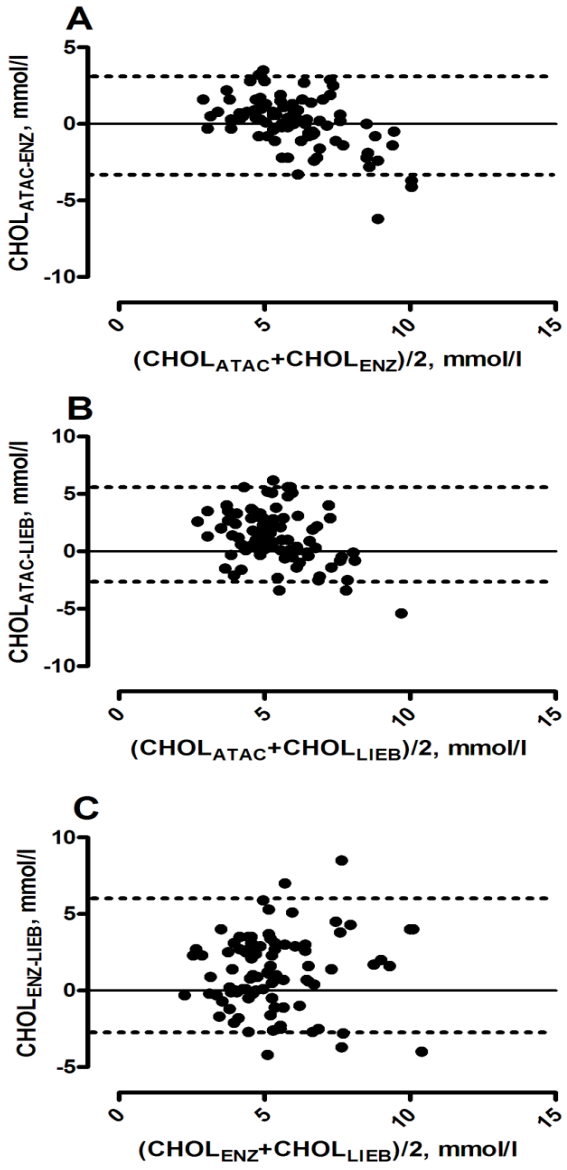

Figure 1: Bland-Altman plots for total cholesterol as determined for 90 patient samples. (A = *agreement between manual enzymatic method and ATAC 8000*; B = *agreement between WHO recommended Liebermann-Burchard manual method and ATAC 8000;* C = *agreement between the manual enzymatic method and the WHO recommended Liebermann-Burchard method*. The 95% confidence limits of the bias are shown as two dotted lines).

ry performance with patient specimens (Ross *et al.*, 1993). This setback is especially true in analyses of total serum cholesterol (Baadenhuijsen *et al.*, 1995). In this approach, the replication experiment was thus, carried out using serum pooled from 350 patient samples since that was likely to have a similar matrix to human serum than bovine or lyophilised serum which for total serum cholesterol could have yielded higher readings due to matrix bias.

To eliminate the bias of comparing two methods which have different principles of operation, the manual enzymatic method, employing the same principle for total cholesterol estimation as the ATAC 8000 autoanalyzer was included in the study. The WHO recommended acceptable limits for CV is 3 – 5% and the National Cholesterol Education Programme (NCEP) Laboratory Standardization Panel recommends that laboratories perform cholesterol analyses with a bias ≤ 3.0% from the true value (reference method).The ATAC 8000® autoanalyzer generated results that were within the recommended CV using the two criteria. The WHO recommended Liebermann-Burchard manual method generated results with CV within the WHO recommended value but above that of NCEP recommended value. The CV for the manual enzymatic method was above the recommended CVs using the two criteria.

The capability index (Cp) which is the ratio of the allowed tolerance to the expanded uncertainty of the assay is highest with ATAC 8000 autoanalyzer as compared to other methods. The higher the Cp, the lower is the risk of jumping the tolerance limits and therefore the higher the quality. The capability index, 1.2 of the manual enzymatic kit method despite the methods poor CV reduces the tendency of the method to fall out of the tolerance.The implication is that the contributing factors, which affect the reproducibility of total cholesterol, were minimised to produce reliable results from the patient samples. Thus, good handling techniques such as accurate pipetting, homogeneity and accurate timing, use of clean cuvettes (specifically glass cuvettes as the acidic nature of the reagent could lead to cloudiness in the plastic cuvettes which would lead

to inaccurate readings), or reading of samples without air bubbles, use of clean glass wares and pipettes et cetera were practiced.

ATAC 8000 furthermore yielded a higher mean value from method comparison using the 90 patient samples, indicating the tendency that the ATAC 8000 would yield consistently higher readings relative to that of the two manual methods. The incidence of interferences with direct cholesterol determinations have been documented (Fasce and Vanderlinde, 1972; Lolekha *et al.*, 2004; Moline and Barron, 1969) and the earlier reports of this method were careful to point out the lack of effects traceable to elevated bilirubin, haemolysis or γ-globulins. Though, the manual method reportedly has considerable serum-clearing effects on lipaemic sera and results are said to correlate very well with that of the Abell method for highly lipaemic sera (Parekh and Jung, 1970). Previous reports have mentioned the depressed cholesterol values displayed by highly lipaemic sera analyzed by a direct manual method (Moline and Barron, 1969).

Performance indices (Pp) which denote the capabilities on one side of the distribution, the side for which the larger proportion of non-conformers will result indicates that, the ATAC 8000 and the WHO reference method are theoretically capable but are practically prone to the slightest drifts or shifts, thus indicating an insufficient quality of the two methods under consideration. The manual enzymatic method however with its performance index of 0.7 shows that the quality of the method is bad and needs to be improved.

The total error for the manual enzymatic method was above that recommended by the NCEP. This observation combined with the bias and the CV indicates the poor precision and accuracy of the manual enzymatic method in this study. The NCEP recommends that clinical laboratories achieve total error ≤ 8.9% on patient specimens (NCEP, 2001). Precision can be improved by adherence to accepted principles of good laboratory practice and quality assurance. Accuracy can be improved by establishment of traceability to the National Reference System for Cholesterol (NRS/CHOL) through a fresh sample comparison with one of the Cholesterol Reference Method Laboratory Network (CRMLN) laboratories.

The study further compared the ATAC 8000® autoanalyzer with the enzymatic method for total serum cholesterol and the Liebermann-Burchard method on 90 patient samples covering a wide range of cholesterol concentrations. The results indicate good agreement between the ATAC 8000® *vs*. the manual enzymatic method and ATAC 8000® *vs*. Liebermann-Burchard method. This study found a poor agreement between the manual enzymatic methods and the Liebermann-Burchard manual method which finding is however, contrary to previous work (Lie *et al.*, 1976) which indicate good agreement.

CONCLUSION

The study showed that the ATAC 8000® was suitable for clinical studies involving the estimation of total cholesterol judging from the results obtained for the CV, Pp and Cp. There was also significant agreement between the result generated by the ATAC® 8000 and the WHO reference method. The manual enzymatic method is inappropriate for quality control work although it employs the same principle as the ATAC 8000, as can be seen from its poor performance index. The Liebermann-Burchard WHO reference method although employing a dissimilar principle of operation with reference to that utilized by the ATAC 8000 autoanalyzer is suitable for quality control analysis as per the results from this study.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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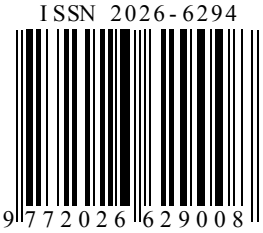

Journal of Medical and Biomedical Sciences (2024) 13(1): 30-36

ORIGINAL ARTICLE

Anti-androgenic activity of Xylopic acid in orchidectomerized rats

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To characterize anti-androgenic properties of xylopic acid (XA) and to elucidate the possible mechanism of the antifertility activity of XA, XA was administered to orchidectomized rats following the Hershberger assay protocol. Thirty male Sprague-Dawley rats were orchidectomized or sham operated at 42 days of age. At 11 days post-castration the rats were weighed and assign to five treatment groups as follows; Group 1 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.), group 2 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 10 mg kg^{-1} of XA orally, group 3 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 30 mg kg^{-1} of XA orally, group 4 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 100 mg kg^{-1} of XA orally, group 5 received only distilled water. The animals were treated daily for 10 days and the weight of the animals taken daily. On the day after the last treatment the rats were necropsied to isolate organs and tissues for study of androgenic or anti-androgenic effects. The endpoints evaluated were the growth/ body weight, and weight of the seminal vesicles plus coagulating glands with fluid, ventral prostate, levator ani plus bulbocavernosus muscle, glans penis, Cowper's glands (bulbourethral glands), and liver all without fixation. XA exhibited anti-androgenic activity by decreasing the weight of these androgen dependent organs.

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Keywords*: Xylopia aethiopica,* Androgenic*,* Hershberger assay , Xylopic acid

INTRODUCTION

Xylopic acid [15-β-acetoxy-(-)-kaur-16-en-19-oic acid], a diterpene kaurane derivative obtained upon extraction of the fruits of *Xylopia aethiopica* with petroleum ether has been shown to cause a reduction in serum testosterone and LH levels as well as a significant reduction in epididymal sperm count, motility and viability in rats (Woode *et al.*, 2012) but no exact mechanism of action was proposed (Woode *et al.*, 2011). Reductions in sperm count and sperm quality as well as reproductive organ weight reduction are mostly associated with androgen antagonist or anti-androgens (Kelce *et al.*, 1995; Thompson and Wilding, 2003). Accessory sex glands and tissues are dependent upon androgen stimulation to gain and maintain weight during or after puberty (Ashby *et al.*, 2000; Yamada *et al.*, 2000).

Reproductive development and function in human and other species may be affected by chemicals that behave as anti-androgens which are linked to the increasing incidence of reproductive cancers and a worldwide decline of semen quality (Toppari, 1996). Some of these chemicals are present in the environment, drinking water and food (Toppari *et al.*, 1996). XA is a major component of the fruits of *Xylopia aethiopica* which is used locally in Ghana as cough remedy, a carminative, a post-partum tonic, and to treat uterine fibroid and amenorrhea (Burkill, 1985; Asekun and Adeniyi, 2004). It is also use as spice in the preparation of most local food in Ghana, Nigeria and Cameroon. Evaluation of the androgen and anti-androgen activity of XA is thus invaluable in our quest to establish the effect of xenobiotic on the reproductive system. The present study was carried out to characterize potential anti-

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androgenic properties of XA and to elucidate the possible mechanism of the antifertility activity of XA in animal modules.

MATERIALS AND METHODS

Plant material

The dried fruits of the *Xylopia aethiopica* [Duna] A. Rich, were obtained from the Botanical Garden, KNUST, Kumasi/Ghana and authenticated in the Department of Pharmacognosy, KNUST, Kumasi, Ghana. A voucher specimen (number FP/08/76) has been deposited in the herbarium of the faculty.

Isolation of Xylopic Acid (X.A)

Xylopic acid was isolated using the method described by Ekong and Ogan (1968). Dry fruits of *X. aethiopica* (0.36 kg) were pulverized and soaked in petroleum ether 40-60 °C for three days. The petroleum ether extract was drained and concentrated using rotary evaporator at a temperature of 50°C. Ethyl acetate (5.0 ml) was added to the concentrate to facilitate crystallization of XA. Crystals formed after three days were washed with petroleum ether 40-60 °C repeatedly. Xylopic acid was purified using recrystallization by dissolving it in ethanol. The resulting solution was filtered and left to stand for three days to recrystallize, yielding 1.41% (5.1 g) of XA with 95% purity. The purity of XA was determined using High Performance Liquid Chromatography (HPLC). The chromatograph consisted of LC-10AT Shimadzu pump with programmable absorbance detector (783A Applied Biosystems) and Shimadzu CR501 chromatopac. Phenomenexhypersil 20 micron C18 200 × 3.20 mm column was used. The mobile phase consisted of methanol and water (9:1) eluted isocratically at 0.5 ml min^{-1}. Portions of 20 µl of a suitable concentration of pure XA were loaded and injected unto the column after dissolving in the mobile phase at 60°C. The eluent was monitored at 206 nm. Portions of the Xylopic Acid Extract (XAE) and XA were loaded and injected. The peak (s) was noted as component(s) of the XAE and XA.

Drugs and chemicals

Pentobarbitone was obtained from the Sigma-Aldrich Inc., St. Louis, MO, USA. Testosterone propionate was a gift from Abeth Consult limited (Kumasi, Ghana).

Hersberger assay

Animals

All experiments were performed with immature Sprague-Dawley rats weighing 60-70 g bought from Noguchi Memorial Institute for Medical Research, University of Ghana, Accra and kept at the Animal House Facility of the Department of Pharmacology, KNUST, Kumasi. The animals were allowed to acclimatize to the laboratory condition (Temperature 24-26°C and 12 hour light-dark cycle) for two week before commencement of the experiment. The rats were allowed free access to solid pellet diet (GAFCO Trading Company, Tema) and water *ad libitum* throughout the study. Prior permission was obtained from the ethical committee of the Pharmacology Department, KNUST. All the animals were treated according to the National Institute of Health Guidelines for the care and use of laboratory animals (NIH, Department of Health and Human Services Publication no. 85-23, revised 1985).

Experimental Procedure

The experiment was carried out according to the Hershberger assay (1953) as modified by Dorfman (1962). Thirty male Sprague-Dawley rats were orchidectomized or sham operated at 42 days of age. The animals were anaesthetized with pentobarbitone and the testes were exteriorized via a midline incision. The testicular blood vessels were clamped and ligated and each testis was removed. The midline musculature was sutured and the skin was auto clipped. The condition of the animals was checked on a daily basis and the clips were removed from the healed wound 7 days after the operation. At 11 days post-castration the rats were weighed and assign to five treatment groups as follows; Group 1 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.), group 2 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 10 mg kg^{-1} of XA orally, group 3 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 30 mg kg^{-1} of XA orally, group 4 received 0.4 mg kg^{-1} day^{-1} of

testosterone propionate (s.c.) plus 100 mg kg^{-1} of XA orally, group 5 (sham operated) received only distilled water. The oral administration of X.A was done 30 minutes after the subcutaneous injection of testosterone propionate. The animals were treated daily for 10 days and the weight of the animals taken daily. On the day after the last treatment the rats were necropsied to isolate organs and tissues for study of anti -androgenic effects. The endpoints evaluated were the growth/body weight, and weight of the seminal vesicles plus coagulating glands with fluid, ventral prostate, levator Ani plus bulbocavernosus muscle (LABC), glans penis, Cowper's glands (bulbourethral glands), and liver all without fixation.

Statistical analysis

Results are expressed as mean ± SD. The significance of difference between the means was determined by one-way analysis of variance (ANOVA) with Newman-Keuls's as post-hoc test. In all statistical tests, a value of $P<0.05$ was considered significant. All analysis was performed using Sigma Plot for Windows, Version 11.0, (Systat Software, Erkrath, Germany; www.systat.com).

RESULTS

Finger print of XAE and XA in TLC and HPLC

The TLC of the extract showed several spots which indicate the presence of several compounds (figure 1a). On the contrary, XA revealed a single spot indicating the presence of a single compound (figure 1b). Several HPLC peaks were observed after loading XAE indicating the presence of several compounds in the fruits as shown in figure 2. A single peak was observed for XA indicating the presence of a single compound (Figure 3) with 95% purity.

Anti-androgenic activity

The body weight of animals that received XA did not differ significantly from vehicle control group and the reference control that received only TP as shown in figure 4. XA administration to orchidectomised testosterone-treated male rats reduced significantly both absolute and relative weight of the following tissues, seminal vesicle (14.4%), prostate (26.6%), Glans penis (22.3%), LABC (7.7%), Cowper's gland (14.6%). The number in parentheses re-

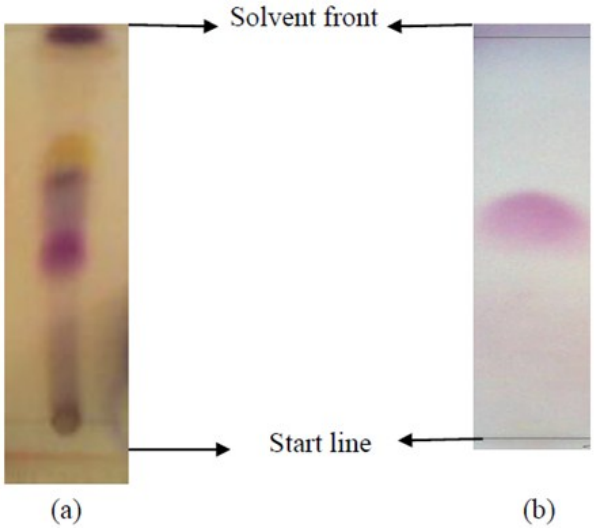

Figure 1: TLC results of (a) extract revealing several spots indicating the presence of several compounds and (b) xylopic acid showing a single spot indicating the presence of a single compound

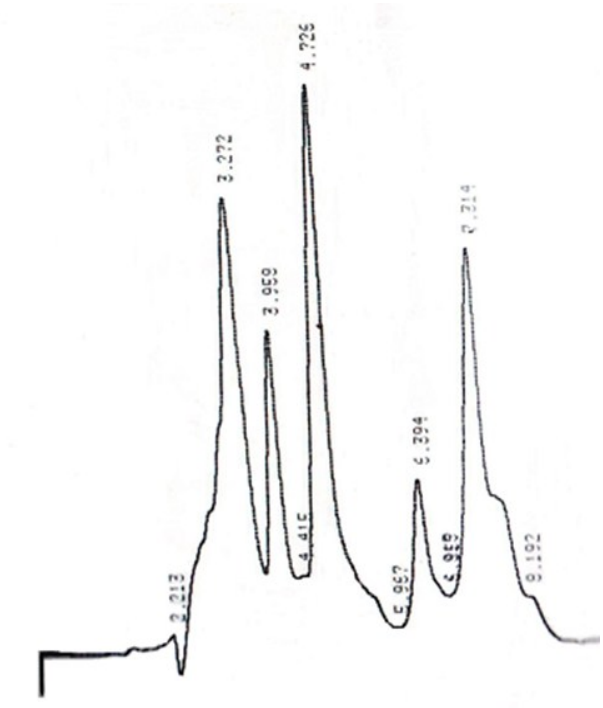

Figure 2: HPLC finger print of the extract showing peaks of the various compounds in the extract.

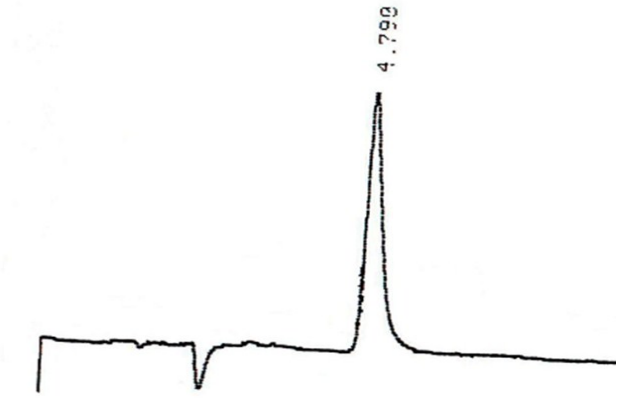

Figure 3: Chromatogram of XA showing a single peak corresponding to the isolated XA.

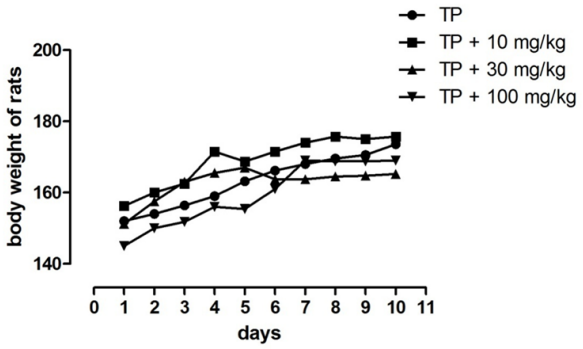

Figure 4: Effect of XA on animal body weight

fers to percentage reduction of absolute weight caused by 10 mg kg^{-1} of XA. The weight reduction were even more pronounce at the middle dose of 30 mg kg^{-1} and at the highest dose of 100 mg kg^{-1} of XA compare to the control and the testosterone propionate treated group as shown in figure 5. However, testosterone administration to orchidec-

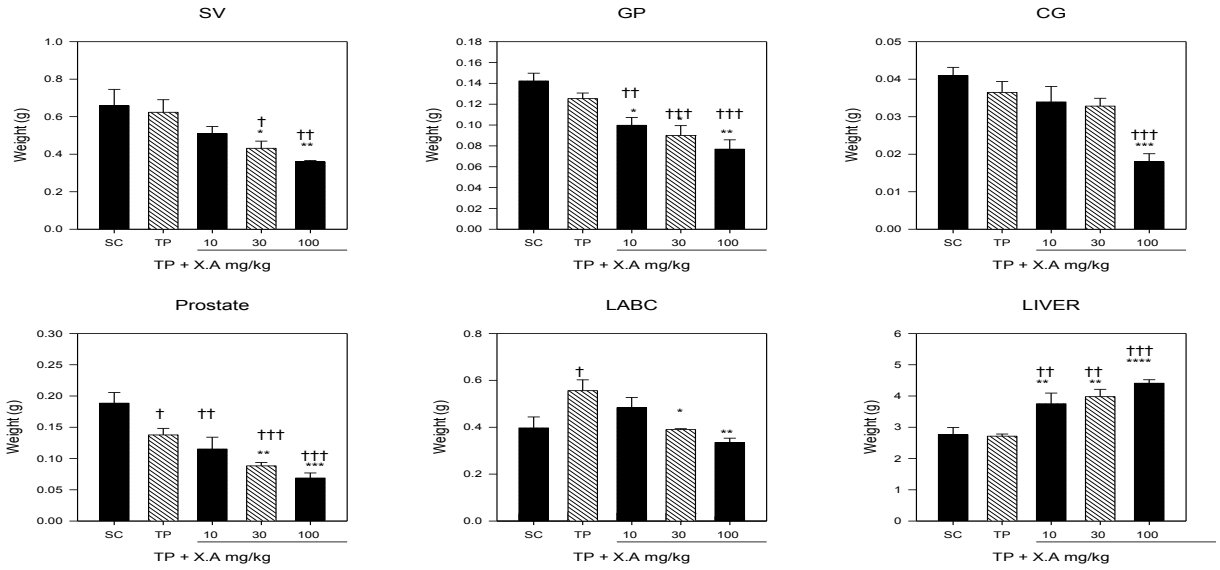

Figure 5: Relative weight of androgen dependent organs. Seminal Vesicles (SV), Glans Penis (GP), Coagulating Gland (CG), Prostate, LevatoraniBulbocavanous Muscle (LABC), Liver from sham castrated and castrated rats treated with Testosterone Propionate (0.4 mg/kg sc) with or without Xylopic Acid (X.A) at doses of 10, 30 and 100 mg/kg given orally. Results are presented as means ± SEM. *P ≤ 0.05, ** P ≤ 0.01, ***P ≤ 0.001 compared to TP treated. (one-way ANOVA followed by Newman-Keuls post hoc); †P ≤ 0.05, ††P ≤ 0.01, †††P ≤ 0.001 compared to sham castrated control rats (one-way ANOVA followed by Newman-Keuls post hoc).

tomised rats increase significantly both absolute and relative weight of the androgen dependent tissues as follows seminal vesicle (7.8%), prostate (4.6%), Glans penis (9.7%), LABC (1.4%), Cowper's gland (6.6%) compare to the vehicle treated animals as shown in figure 5. Subcutaneous administration of TP had the expected stimulatory effect on the androgen dependent tissues as stated above. Co-administration of XA with TP, as observed essentially abolished the stimulatory effects of the standard androgen on the tissues. The weight of the liver in rats receiving TP plus various doses of XA was significantly increased in a dose dependent manner compare to control and TP administered rats as shown in figure 5.

DISCUSSION

Various xenobiotics and naturally occurring compounds have been found to disrupt the endocrine system of animals (Toppari *et al*., 1996). Reduction in androgen-dominance to oestrogens and interference with androgen action are apparent mechanisms causing demasculinization and fertility decline in males (McKinnell *et al*., 2001; Williams *et al*., 2001; Rivas *et al*., 2002). In the present study, when XA. was administered to male rats orally, it exhibited anti-androgenic activity as seen by the significant decrease in the weight of the seminal vesicles, ventral prostate, LABC, glans penis, and the Cowper's gland, as these organs are dependent on androgens. This anti-androgenic action further support earlier report (Woode *et al*., 2012) which showed that administration of XA to adult male rats resulted in a significant reduction in sperm count, motility, and viability and significantly increase abnormal sperm morphology as well as decreases the number of Leydig cells and the seminiferous tubular diameter.

Anti-androgens may exhibit their activity both peripherally on androgen-dependent tissues and by feedback action at a central site (Mainwaring, 1977; Neumann *et al*., 1977; Moguilewsky and Raynaud, 1979; Raynaud *et al*., 1979; Neumann, 1985). XA may thus be competing for the peripheral androgen receptors and thus inhibit the effect of endogenous or exogenous androgens. Centrally, XA might be inhibiting gonadotropin secretion and thereby diminish testosterone production by the gonads (Neumann *et al*., 1970; Neri, 1977; Hans, 2007). Additionally, XA could also be an inhibitior of 5α-reductase, an enzyme located in tissues such as the prostate, seminal vesicle, epididymis, skin and sebaceous glands. Such inhibitors reduced the conversion from testosterone to 5α-dihydrotestosterone (DHT). Inhibition of 5α-reductase provides a selective approach to androgen deprivation in DHT-target tissues, such as the prostate (Hans, 2007).

CONCLUSION

In conclusion, XA exhibited anti-androgenic by reducing the weight of the androgen dependent organs possible by blocking androgen receptors which prevents androgens from binding to them and suppresses luteinizing hormone which in turn reduces testosterone levels thus suppressing the actions of testosterone and its metabolite dihydrotestosterone on tissues. The results thus confirm the earlier report of antifertility activity of XA in male rats.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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