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REVIEW ARTICLE

Probiotics in colon cancer prevention

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ABSTRACT

Probiotics are live, selected microbial strains that have a beneficial effect on the human body and when introduced into the body, they colonize in the digestive tract, especially in the large intestine, exerting a beneficial effect on the health of the host. The microbial strains, so that they can be included in the probiotics, must be thoroughly tested and meet several conditions. These microorganisms multiply in the gastrointestinal tract and are competitive for pathogenic microorganisms that cause infection. Probiotic bacteria are found in natural yogurts, sour milk, sauerkraut, pickled cucumbers. Many studies show a positive correlation between the consumption of probiotics and the risk of developing certain cancers. Probiotics are most likely to reduce the risk of developing colorectal cancer.

Keywords: Probiotics; Colon; Cancer; Prevention; Bacteria.

INTRODUCTION

Among the microorganisms of the natural microflora, bacteria with probiotic properties play an enormous role. These bacteria have a beneficial effect on the functioning of the human body, both local and systemic [1-3]. For a microbial species to be considered as probiotic it must be fully understood. Detailed studies of its impact on the human and animal organism last even several years. Clinical procedures, during which a given species of a microorganism is classified as probiotic, consist of three phases: strain's safety assessment, checking the effectiveness of the strain and verification of the strain's effectiveness on a large number of people and comparison of the effects of treatment with standard therapy [2, 4, 5].

Moreover, the qualified strain should meet the following criteria: it must come from the natural healthy microflora of the human intestine, it is an absolute or relative anaerobe, it should belong to a specific type and species that has been assigned to it using molecular methods, it should be resistant to acidic pH of gastric juice, bile salts and digestive enzymes, its positive effect should be scientifically confirmed and must maintain, it cannot show pathogenic or toxic properties, its all properties during processing and storage [6-8].

CHARACTERISTICS AND FUNCTION OF PROBIOTICS

The characteristics of the probiotics are strain-dependent. Positive properties of one microorganism will not necessarily occur in another, even closely related one. Probiotics activity may always refer only to one tested strain, not to the species, genus or to all lactic bacteria [2, 4, 5]. The composition of the probiotic may contain single strains of lactic acid bacteria (*Lactobacillus* spp., *Streptococcus* spp.), yeast (*Saccharomyces* spp.) or lactic acid bacteria in combination with yeast strains. Probiotic bacteria are found in fermented foods and can also be found in pharmaceutical preparations [9].

Table 1. Classification of probiotics.

Genus	Species
Lactobacillus spp.	*acidophilus, brevis, casei, delbrueckii gasseri, fermentum, johnsonii, lactis, paracasei, plantarum, reuteri, rhamnosus*
Bifidobacterium spp.	*adolescentis, animalis, bifidum, breve, infantis, lactis, longum, thermophilum*
Bacillus spp.	*coagulans*
Streptococcus spp.	*thermophilus*
Enterococcus spp.	*faecium*
Saccharomyces spp.	*cerevisiae*

The advantages of probiotics are used in many fields, e.g. they restore the natural intestinal microflora after the antibiotic treatment, they are used for the production of functional food and for the preservation of food products [10].

To notice the beneficial effect of probiotics on the body, they should be consumed for a long time so that the positive microflora persists at a high level. The composition of the intestinal microflora depends mainly on the type and composition of the food consumed and the age of the person. Intestinal flora is affected by past infections, antibiotic therapy, availability and composition of substrates for microflora growth, interactions with the immune system, intestinal pH, bacterial metabolites, intestinal status, as well as place of residence and lifestyle [10-12].

Bacteria with beneficial effects classified as probiotics are usually heterogeneous gram-positive, catalase-negative cocci or rods of the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Oenococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Bifidobacterium* and rare *Weissella*, *Carnobacterium*, *Tetragenococcus*, *Vagococcus* etc. All of them are able to carry out the anaerobic fermentation of saccharides. They produce lactic acid at the level of 0.6% to 3.0%, during the fermentation process.

They produce L (+) lactic acid or D (-) lactic acid. If they only produce this acid, they are classified as homofermentative bacteria. However, when they also produce acetic acid, ethanol, carbon dioxide, succinate and formate in addition to lactic acid derivatives, they are heterofermentative bacteria. The natural habitat for the occurrence of lactic acid bacteria is: alimentary canal of humans and animals, milk, plants, mucous membranes of the oral cavity and reproductive organs [8, 13].

Numerous studies confirm the beneficial effect of probiotics on human health, which became the basis for their use both in the prevention and treatment of many diseases [1, 2].

PROBIOTICS AND COLON CANCER

Probiotics have a positive effect on intestinal epithelial cells - colonocytes. They provide them with 70% of the energy that is needed to regenerate the intestinal wall in the case of pollutants from the environment. The most important role is played by bacteria that bind to the adhesive receptors in the gastrointestinal tract using

fimbriae. Probiotic bacteria without fimbria must be delivered in larger quantities with food. An important feature of probiotics is also multiplication in the large intestine [14].

In the gastrointestinal tract of an adult, microflora constitutes over 1000 different species of microorganisms [1, 2]. Among them are lactic bacteria, which play an important role in delaying the process of colon cancer formation, most likely affecting metabolic, immune and protective functions. Their amount may increase in the large intestine after ingestion of food containing probiotics. Additional beneficial effects indicated by probiotics are alleviation of lactose intolerance, increasing the humoral immune response, biotransformation of isoflavone phytoestrogen to reduce postmenopausal symptoms, and lowering serum cholesterol [15-17].

Probiotics have an inhibitory effect on the process of carcinogenesis. This is influenced by the ability to reduce harmful bacteria such as *Clostridium*, *Peptostreptococcus* and *Staphylococcus*. Probiotic bacteria affect the inhibition of β-glucoronidase, β-glucosidase and nitroreductase produced by pathogenic bacteria.These are pro-carcinogenic faecal enzymes that are responsible for the growth of colon cancer cells. Probiotics also destroy carcinogens such as nitrosamines and their precursors, and also act destructively on nitroreductase, which is involved in the synthesis of nitrosamines. They positively affect the immune response, increase and development of harmful intestinal microflora, are responsible for the production of antimutagenic substances and the production of lactic acid, which stimulates apoptosis, inhibits the conversion of bile salts into secondary bile salts [18].

Some of the bacteriocins produced by probiotic bacteria are cytotoxic for cancer cells comparing to healthy cells. Bacteriocins, which are cationic, hydrophobic peptides, bind to a negatively charged cell membrane of tumor cells. The cause of this selective binding to the membrane of tumor cells is also due to the difference in the fluidity of their membrane and the greater number of microvilli comparing to normal cells. The bacteriocins cytotoxicity mechanisms include the induction of apoptosis and depolarization of the cell membrane leading to changes in permeability [19, 20].

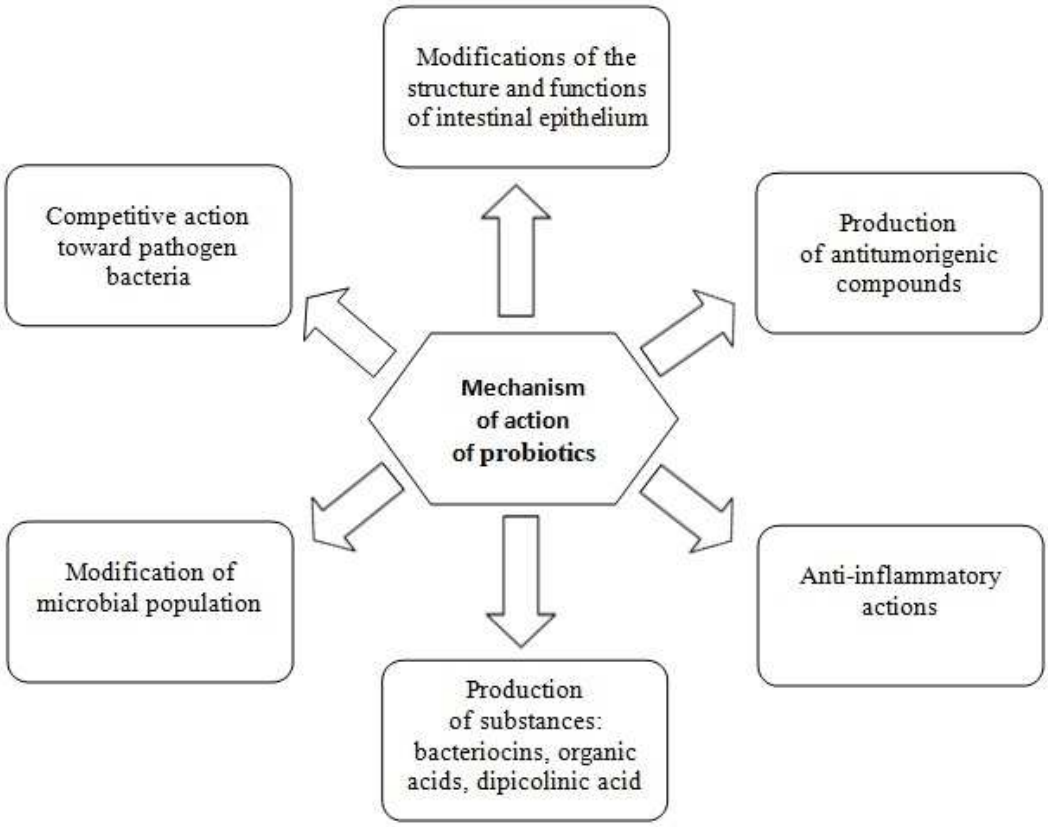

Figure 1. Ways act probiotics.

Colorectal cancer is the second cancer in Poland with the highest mortality [7, 8]. Every year, almost 16,000 new cases are diagnosed. Cancer risk factors are hereditary and environmental factors. Hereditary factors include familial polyposis, hereditary non-arterial colon cancer, Lynch I and II syndromes and ulcerative colitis. Environmental factors include pollution, exposure to certain chemicals, consumption of high-fat low-fiber diets and lack of physical activity [21, 22].

In animal and human studies on the effect of probiotics on factors predisposing to colorectal cancer, researchers investigated the increase of enzymes activity that activate carcinogens, increase the amount of proto-oncogenic chemicals in the colon, or alter populations of certain types or species of bacteria [23-31]. Many studies have shown that factors that can influence the occurrence of colorectal cancer are positively affected by the consumption of certain probiotics [23, 25, 26]. However, these studies do not show a causal relationship to the development of colorectal cancer and are circumstantial. Studies that directly examine the causal relationship are animal studies. In people with colorectal carcinomas a significant disturbance of the intestinal microflora is observed, which plays an important role in the pathogenesis of this common disease. Early studies have shown that the metabolic activity of intestinal microorganisms leads to the production of carcinogens or pro-carcinogens in the large intestine [32]. Under the influence of microflora, the activation of procarcinogens delivered to the body with the diet and biliary excreted to the large intestine, carcinogen synthesis and enzymatic modification of carcinogenic compounds detoxified in the liver are caused [32].

Animal studies show that only 20% of animals free from the microorganisms have chemically induced colon cancer. In animals with microflora, this value was 93%. Cytochrome P450 studies at the molecular level have shown that some of the P450s are also active carcinogens. Epidemiological studies show a higher risk of colorectal cancer in people with high CYP1A2 activity. The effect on this cancer in humans is mediated by the metabolic activation of food-borne heterocyclic amines that occurs via N-oxidation followed by O-acetylation with N-acetoxyarolamine formation, which binds to DNA to form carcinogenic adducts from DNA. These steps are catalyzed by the hepatic cytochrome CYP1A2 and acetyltransferase-2 (NAT-2) respectively [33]. Probiotics such as *Bifidobacterium* produce metabolites that may affect the function of P450 and cause the conversion of azoxymethane to the carcinogenic factor. These tests, along with experiments carried out by Reddy et al. suggest that the probiotic can affect the development of colon cancer. They showed in studies that stimulated growth of *Bifidobacterium* in the colon of rats may lead to inhibition of colon cancer, and suggested that the effect on the foci and the number of crypts in the large intestine has the effect of *Bifidobacterium*, which inhibit the growth of *E. coli* bacteria by lowering the pH. Reducing the amount of these microorganisms can also affect the modulation of bacterial enzymes, such as beta-glucuronidase, which can transform pro-carcinogenic factors into cancerogenic ones [23].

Studies on the antimutagenicity of probiotics show that antimutagenic substances can be found in the cellular envelope of the bacterial cell wall [35]. During in vitro studies on colon cancer cells isolated from mice, *Bifidobacterium infantis* was found to inhibit the activity of this tumor.

These studies suggest that mutagens bind to the cell wall of probiotics and that *Bifidobacterium* binds to the final carcinogen methylazoxymethanol and the mutagen-carcinogen 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole, thus removing it from the faeces, and then minimizing its absorption into the lumen of the intestine [24, 35].

The conducted research shows that a significant role in bacterial antimutagenicity may play the stage of their growth. In the phase of linear growth, significant antimutagenic activity is achieved, reaching the maximum level, which then decreases in the stationary growth phase [36, 37].

Baricault et al. performing an in vitro study of HT-29 colon carcinoma cells to which fermented milk was added, concluded that a protective action of probiotics is based on the change of cancer cells differentiation process. The milk was prepared to fermentation using single strains of *Lactobacillus helveticus*, *Bifidobacterium*, *L. acidophilus* or mixture of *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. On the basis of the research, it was shown that 10-50% of HT-29 cells under the influence of fermented milk inhibited their growth. Subsequent studies have shown that the activity of specific markers responsible for the differentiation of HT-29 cells such as dipeptidyl peptides has been increased [38].

Singh et al. performing analyzes on male F344 rats, evaluated the effect of *Bifidobacterium longumna* on the development of colorectal cancer. The obtained results showed that the administration of lyophilized *B. longum* cultures in food products inhibited the incidence of colon cancer and also reduced the tumor volume. Bacteria *B. longum* also inhibited azoxymethane induced cell proliferation by lowering ornithine decarboxylase (ODC) activity [34]. Ornithine decarboxylase participates in polyamine biosynthesis, which is responsible for the proliferation, differentiation and macromolecular synthesis of cells. The increase in ODC activity correlates with the growth of colorectal adenoma, which indicates a hyperproliferative state of the colonic mucosa [39]. In the conducted studies, it was also shown that the anti-tumor effect is affected by the reduced expression of *ras-p21* oncoprotein. Activation of *ras* proto-oncogenes may induce a malignant phenotype in colon cells [40]. The malignant potential of *ras* genes is related to the mutation in codons 12, 13 or 16 [41].

The study suggests that the administration of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* ssp. *lactis* Bb12, reduces the risk of colorectal cancer as a result of the reaction with endogenous or exogenous carcinogens [42]. Research results of Witkin et al. showed a correlation between the presence of *Lactobacillus* ssp. and *Eubacterium aerofaciens* strains, and a reduced risk of colon cancer [43]. Moreover, epidemiological studies in Finland have shown that high intake of probiotic products resulted in a reduction in the incidence of colorectal cancer despite high fat intake [44].

CONCLUSIONS

Studies that have been published so far do not show clearly that probiotics can prevent colorectal cancer. Epidemiological research is contradictory. Some of them show a lower risk of colon cancer in people consuming probiotics, but there are also those that did not show a relationship the consumption of fermented milk products and the risk of developing disease. Furthermore, not all protective activities of colostoma probiotics were confirmed during in vivo tests. Inconsistent data from these studies may be related to the complexity of carcinogenesis, experimental design, variability of probiotic strains and changes in cancer stages. The onset of cancer related processes in the body occurs many years before the diagnosis, while the colonization of the digestive tract by the intestinal microflora is a very dynamic process, changing under the influence of pH, differences in the content of nutrients and oxygen. Despite the many different results obtained during probiotic studies, further studies are needed to confirm their clinical efficacy.

AUTHORS' CONTRIBUTION

MW, AB, BJ, AP: Writing of the manuscript; BC: Conception and design. All authors read and approved the final manuscript.

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RESEARCH ARTICLE

Effect of *Aloe vera* gel on some haematological parameters and serum electrolytes in high salt loaded Wistar rats

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ABSTRACT

This study investigated the effect of *Aloe vera* gel on some haematological parameters and serum electrolytes in high salt loaded rats. Twenty (20) male Wistar rats (180-250 g) were randomly assigned into 4 groups (n=5): Control-received 0.2 ml normal saline; *Aloe*-received 600 mg/kg of *Aloe vera* gel orally once daily; Salt-fed (SF) received high salt diet (8% NaCl in feed + 1% NaCl in H_2O); Saltfed-treated (SF+*Aloe*) received high salt diet + *Aloe vera* gel. All groups had access to rat feed and water throughout the duration (six weeks) of treatment. Blood samples were collected from each animal via cardiac puncture for analysis. Red blood cell (RBC) count, haemoglobin (Hb) concentration and packed cell volume (PCV) were significantly ($p<0.05$) increased in SF and SF+*Aloe* groups compared with control and *Aloe* groups. Total white blood cell count was significantly ($p<0.001$) decreased in SF group compared with control and *Aloe* groups and increased ($p<0.001$) in SF+*Aloe* group compared with SF group. Neutrophil and lymphocyte counts were significantly increased and decreased respectively in SF+*Aloe* group compared with control ($p<0.01$), *Aloe* ($p<0.05$) and SF ($p<0.001$) groups. Na^+, K^+ and Cl^- concentrations were significantly increased in SF and SF+*Aloe* group compared with control and *Aloe* groups. HCO_3^- concentration was significantly increased in *Aloe* and SF+*Aloe* groups compared with control. High salt diet (HSD) caused alterations in red cell indices and posed threat to the immune system of rats. *Aloe vera* could not reverse these alterations but exhibited an immune-stimulatory effect. Both *Aloe vera* and HSD caused electrolyte imbalance.

Keywords: *Aloe vera* gel; Electrolyte; Haemoglobin; High salt diet; Red blood cell; White blood cell.

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INTRODUCTION

Salt is an essential component of life. It is composed chemically of sodium (Na^+) and chloride (Cl^-). It is used as ingredient in food and also useful in areas such as water conditioning processes, ice control and road stabilization, preservation of meat and fish and production of some other chemicals [1]. Because of its abundance and essentiality to life, salt has been designated as the fifth element being equated with earth, air,

water and fire [2, 3]. Consumption of 5 g of salt/day is the World Health Organisation (WHO)'s recommendation for adults while for children; this amount should be reduced based on the energy requirement of children relative to those of adults [4]. Excessive intake of salt is dangerous to health. It has demonstrated several deleterious effects in the body. It has been implicated in hypertension [5], kidney damage [6], osteoporosis [7], liver toxicity and fibrosis [8, 9] and has been reported to decrease plasma concentration and urinary excretion of nitrates [10]. High salt diet (HSD) ingestion has also been reported to increase the severity of asthma and is strongly related to gastric carcinoma [11]. The deleterious effects of high salt diet ingestion have been associated with oxidative stress [12-14].

Aloe vera, a plant of the family, Asphodelaceae has been reported to have the ability to combat oxidative stress [15]. It can be separated into two basic products: gel and latex. The gel is the transparent mucilage that is gotten from the pulp of the leaves while the latex (juice) is the bitter yellow exudate that is gotten from the outer skin of the leaves [16]. *Aloe vera* has demonstrated several therapeutic effects. It has been reported to inhibit growth of tumor in mice [17], alleviate respiratory tract disorders [18] and cardiovascular system disorders [19, 20]. It is also effective in treating radiation-induced dermatitis [21] and have anti-atherogenic [22], anti-ulcer [23] and immune-stimulatory [17] effects. *Aloe vera* has also been reported to reverse haemostatic derangement caused by salt loading [24].

Studies have recorded the effect of *Aloe vera* on blood physiology [22, 25-29]. But no study has recorded the effect of *Aloe vera* gel on haematological parameters and serum electrolytes of Wistar rats fed on HSD. In view of the desirability and expedience of cheaper remedies to combat complications associated with high salt intake, coupled with the therapeutic efficacies of *Aloe vera* and the paucity of information on the effect of *Aloe vera* gel on blood parameters and serum electrolytes following HSD, this study was therefore carried out to investigate the effect of *Aloe vera* gel on some haematological parameters and serum electrolytes in high salt loaded rats.

MATERIALS AND METHODS

Experimental animals

Twenty male Wistar rats (180-250 g) bought from the Department of Agriculture, University of Calabar, Nigeria were employed in the study. The animals were handled according to standard principles [30]. They were kept in properly ventilated metabolic cages in the animal house of Department of Physiology, University of Calabar and exposed to 12/12 hours light/dark cycle. The rats were given rat feed and water *ad libitum* and allowed to explore their new habitat for seven days before commencement of experiment.

Preparation of *Aloe vera* gel extract

Aloe vera plant was obtained from a garden in the University of Calabar. The leaves were being certified by the chief Hebarium in the Department of Botany, University of Calabar. The fresh leaves were thoroughly washed with tap water to remove dirt. Surgical blades were used to cut the base and apex of the leaves. The leaves were then sliced open along the margin to reveal the transparent mucilage which was then scooped into a beaker using a spatula. The mucilage was further processed by blending for 20 minutes in an electric blender and a greenish gel-like liquid obtained. This liquid was kept for 20 minutes to settle and later sieved using Whatman filter paper to obtain a particulate-free gel [31]. The *Aloe vera* extract was refrigerated (4-6°C) for 3 days after use each day.

Preparation of high salt diet and drinking water

High salt diet containing 8% of sodium chloride was prepared using a standard diet containing 0.3% sodium chloride as described by Obiefuna and Obiefuna [32].

Experimental design and extract administration

Twenty male Wistar rats were randomly assigned into four (4) groups (n=5) thus: Group 1 (control) received normal rat feed and water. Group 2 (*Aloe*) received 600 mg/kg of *Aloe vera* gel orally once daily. Group 3 (salt-fed) received high salt diet (8% NaCl feed + 1% NaCl drinking water) and group 4 (salt-fed-treated [SF+*Aloe*]) received same as group 2 + high salt diet. All groups had access to rat feed and water throughout the duration (six weeks) of the experiment.

Collection of blood samples

At the end of the 6 weeks, the rats were sacrificed under chloroform anaesthesia (3.5%) and blood samples collected via cardiac puncture using 5 ml syringes with 21G needles into sample bottles and pre-labelled ethylenediaminetetraacetate (EDTA) vials for measurement of serum electrolytes concentration and haematological parameters respectively. The samples in the EDTA vials were gently agitated to ensure uniform spread of EDTA.

Measurement of haematological parameters

Haematological parameters were measured using automated cell counter (Coulter Electronics, Luton, Bedfordshine, UK) having standard calibrations in line with the instructions of the manufacturer as previously used by Archibong et al. [33]. Parameters measured were: RBC count, PCV, Hb concentration, MCV, MCH, MCHC, WBC count, lymphocyte count and neutrophil count.

Measurement of serum electrolytes concentration

The collected blood samples were allowed for 1 hour to clot and retract. Blood in the sample bottles were centrifuged at 300 rpm at room temperature for 15 minutes using a bucket centrifuge machine (B-Bran Scientific and Instrument Company, England) and serum was obtained. The serum obtained was then used to determine serum Na^+, K^+, Cl^- and HCO_3^- levels using ion-selective electrolyte analyser (Biolyte 2000/ BioCare Corporation, Hsinchu 300, Taiwan).

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Data were analysed using Computer software, SPSS (version 21). Statistical measure used was one-way analysis of variance (ANOVA) along with post hoc multiple comparison test (least square difference). $P<0.05$ was the criterion for statistical significance.

RESULTS

Comparison of haematological parameters in the different experimental groups

RBC count, haemoglobin concentration and PCV

Table 1 shows RBC count ($x10^6$ cell/µl), Hb concentration (g/dl) and PCV (%) for control, *Aloe*, Salt-fed (SF) and saltfed-treated (SF+*Aloe*) groups. RBC count was significantly increased in SF and SF+*Aloe* groups compared with control ($p<0.001$) and *Aloe* groups ($p<0.01$ and $p<0.001$, respectively). Hb concentration was significantly ($p<0.05$) increased in SF and SF+*Aloe* groups compared with control and *Aloe* groups. PCV was also significantly ($p<0.05$) increased in SF and SF+*Aloe* groups compared with control and *Aloe* groups. RBC count, Hb concentration and PCV were not significantly different between control and *Aloe* groups.

Table 1. Comparison of RBC, Hb concentration and PCV in the different experimental groups.

Parameter	Control	*Aloe*	SF	SF+*Aloe*
RBC Count (x10^6 cell/µl)	4.58±0.09	4.92±0.12	5.52±0.16^{***,b}	5.78±0.19^{***,c}
Hb conc (g/dl)	16.36±0.34	16.53±0.58	17.62±0.34^{*,a}	17.62±0.34^{*,a}
PCV (%)	49.20±1.02	49.67±1.74	53.00±1.74^{*,a}	53.00±1.96 ^{*,a}

Values are expressed as mean ± SEM, n = 5.
*p<0.01, ***p<0.001 vs control; a = p<0.05, b = p<0.01, c = p<0.001 vs *Aloe.*

Red cell absolute values

Table 2 shows MCV (fL), MCH (pg) and MCHC (g/dL) for control, *Aloe*, SF and SF+*Aloe* groups. MCV and MCH were significantly decreased in SF (p<0.001) and SF+*Aloe* (p<0.001) groups compared with control. MCV was significantly (p<0.005) decreased in *Aloe* group compared with control. MCV was significantly decreased (p<0.01) in SF+*Aloe* group compared with *Aloe* group. MCH was significantly decreased (p<0.01) in SF and SF+*Aloe* groups compared with *Aloe* group. There was no significant difference in MCHC in the different experimental groups.

Table 2. Comparison of red cell absolute values in the different experimental groups.

Parameter	Control	*Aloe*	SF	SF+*Aloe*
MCV (fL)	107.45±1.53	100.99±0.85*	96.22±0.85***	92.00±0.54^{***,b}
MCH (pg)	35.73±0.50	35.62±0.97	31.99±0.28^{***,b}	30.58±0.18^{***,b}
MCHC (g/dL)	33.25±0.02	33.29±0.03^{ns}	33.24±0.02^{ns}	33.23±0.01^{ns}

Values are expressed as mean ± SEM, n = 5.
ns = not significant; *p<0.01, ***p<0.001 vs control; b= p<0.01 vs *Aloe*.

White blood cell indices

Table 3 shows TWBC (x10^3 cell/µl), NEUT (%) and LYM (%) for control, *Aloe*, SF and SF+*Aloe* groups. TWBC was significantly (p<0.001) decreased in SF group compared with control and *Aloe* groups and significantly (p<0.001) increased in SF+*Aloe* group compared with SF group. NEUT count was significantly increased in SF+*Aloe* group compared with control (p<0.01), *Aloe* (p<0.05) and SF (p<0.001) groups. LYM count was significantly decreased in SF+*Aloe* group compared with control (p<0.01), *Aloe* (p<0.05) and SF (p<0.001) groups. There was no significant difference in NEUT and LYM count between control, *Aloe* and SF groups.

Table 3. Comparison of white blood cell indices in the different experimental groups.

Parameter	Control	*Aloe*	SF	SF+*Aloe*
TWBC (x10^3 cell/µl)	8.84±0.05	8.63±0.12	3.84±0.10^{***,c}	9.08±0.46^{z}
NEUT (%)	62.60±2.44	70.17±2.13^{ns}	55.80±3.40^{ns}	80.60±1.89^{**,a,z}
LYM (%)	36.20±2.29	28.50±2.08^{ns}	42.80±3.27^{ns}	18.20±2.24^{**,a,z}

Values are expressed as mean ± SEM, n = 5.
ns = not significant vs control; **p<0.01, ***p<0.001 vs control; a= p<0.05, c= p<0.001 vs *Aloe*; z= p<0.001 vs SF.

Comparison of serum electrolytes concentration in the different experimental groups

Table 4 shows serum concentrations of Na^+ (mmol/l), K^+(mmol/l), Cl^- (mmol/l) and HCO_3^- (mmol/l) for control, *Aloe*, SF and SF+*Aloe* groups. Na^+ concentration was significantly increased in *Aloe* ($p<0.01$), SF ($p<0.001$) and SF+*Aloe* ($p<0.001$) groups compared with control. It was also significantly increased in SF ($p<0.001$) and SF+*Aloe* ($p<0.01$) groups compared with *Aloe* group and significantly ($p<0.001$) decreased in SF+*Aloe* group compared with SF group.

K^+ concentration was significantly increased in SF ($p<0.05$) and SF+*Aloe* ($p<0.001$) groups compared with control and *Aloe* groups. K^+ was also significantly ($p<0.001$) increased in SF+*Aloe* group compared with SF group. There was no significant difference in K^+ concentration between control and *Aloe* groups.

Cl^- concentration was significantly ($p<0.001$) increased in all experimental groups compared with control. It was significantly ($p<0.01$) increased in SF group compared with *Aloe* group and decreased ($p<0.05$) in SF+*Aloe* group compared with SF group.

HCO_3^- concentration was significantly increased in *Aloe* ($p<0.05$) and SF+*Aloe* ($p<0.001$) groups compared with control. It was also significantly increased in SF+*Aloe* group compared with *Aloe* ($p<0.05$) and SF ($p<0.001$) groups.

Table 4. Comparison of serum electrolytes concentration in the different experimental groups.

Parameter	Control	*Aloe*	SF	SF+*Aloe*
Na^+	140.25± 0.63	143.00± 0.41**	150.00± 0.82^{***,c}	145.75± 0.48^{***,b,z}
K^+	4.45± 0.06	4.43± 0.05	4.68± 0.05^{*,a}	5.58± 0.11^{***,c,z}
Cl^-	94.75± 0.48	98.25± 0.85***	101.00± 0.58^{***,b}	98.50± 0.29^{***,x}
HCO_3^-	20.50± 0.29	22.50± 0.96*	21.00± 0.58	25.00± 0.58^{***,a,z}

Values are expressed as mean ± SEM, n = 5.
*p<0.05, **p<0.01, ***p<0.001 vs control; a= p<0.05, b= p<0.01, c=p<0.001 vs *Aloe*; x= p<0.05, z= p<0.001 vs SF.

DISCUSSION

High salt intake (HSI) has been reported to impact negatively on various cells and tissues of the body [8-11]. *Aloe vera* has been shown to protect cells from damages caused by various toxic substances [21-24].This study investigated the effect of *Aloe vera* gel on some haematological parameters and serum electrolytes in high salt loaded Wistar rats.

Results from this study show that RBC, Hb concentration and PCV were significantly increased in salt-fed (SF) and saltfed-treated (SF+*Aloe*) groups compared with control and *Aloe* groups. Our results for RBC, Hb concentration and PCV are consistent with Ofem et al. [28] who reported that high salt intake and co-administration of salt and *Aloe vera* gel increased RBC count, Hb concentration and PCV in rats. But in their study, Hb concentration was not significantly affected by HSI. The increase in RBC and PCV could be due to dehydration caused by high salt loading. This increase is a predisposing factor to hypertension as it could possibly lead to increase blood viscosity. *Aloe vera* gel was unable to reverse this increase caused by HSI as the group that received salt diet + *Aloe vera* gel also had elevated RBC count and PCV. PCV increased following the increased RBC count. The increase in Hb concentration is probably due to the increase in RBC count or stimulation of haeme biosynthesis during the process of erythropoiesis.

MCV was significantly decreased in all treatment groups compared with control. It was also significantly decreased in SF+*Aloe* group compared with *Aloe* group. The decreased MCV indicates that the RBCs became microcytic following administration of *Aloe vera* gel and salt separately and in combination. The effect was greatest when salt and *Aloe vera* gel were co-administered as observed in the SF+*Aloe* group (Table 2). This

decrease in MCV could be attributed to dehydration caused by high salt loading. Our result for MCV contradicts Ofem et al. [28] who reported that high salt loading and co-administration of salt diet and *Aloe vera* gel did not significantly alter MCV. MCH was significantly decreased in SF and SF+*Aloe* groups compared with control and *Aloe* groups. The decrease in MCH in these groups indicates microcytic hypochromic anaemia caused by high salt diet. This decrease in MCH can be attributed to the HSI since *Aloe vera* gel alone did not cause any significant effect on Hb concentration and MCH. It is likely that the salt loading suppressed the synthesis of iron which resulted in microcytic RBCs and hence decreased Hb concentration. *Aloe vera* at the administered dose was unable to prevent this effect. Our result for MCH in SF group is consistent with Ofem et al. [28]. MCHC was not significantly different between the experimental groups.

TWBC count was significantly decreased in SF group compared with control and *Aloe* groups. NEUT count was also decreased although non-significantly in SF group compared with control and *Aloe* groups. This is contrary to Ofem et al. [28] who reported significant increase in TWBC count following salt loading. The decrease in TWBC count in our study indicates the suppression of the defense mechanism and immune system of rats due to salt loading. *Aloe vera* gel however demonstrated a protective effect on the defense mechanism as TWBC count was significantly increased in SF+*Aloe* group compared with SF group and NEUT count significantly increased in this group compared with other groups. LYM count was increased although not significant, in SF group compared with control and *Aloe* groups. This increase could be due to agitation of the immune system following salt loading. LYM count was significantly decreased in SF+*Aloe* group compared with other groups. This could be a demonstration of immune-stimulatory effect of *Aloe vera*. *Aloe vera* had been previously reported to have immune-stimulatory effect [17].

Serum electrolytes play a contributory role in body fluid homeostasis and are important regulators of neuromuscular activities [34]. In addition to altered hormonal status, dietary habit is a factor that causes hormonal imbalances [35]. Results from this study show alterations in serum electrolytes concentrations. Na^+ concentration was significantly increased in all experimental groups compared with control. It was also significantly increased in SF group compared with *Aloe* group and decreased in SF+*Aloe* group compared with SF group. The increase in Na^+ concentration in SF group is consistent with earlier reports that salt loading leads to elevation of serum Na^+ concentration [36]. Salt ingestion increases the osmolarity of body fluids. This stimulates the taste centers to increase water intake and the posterior pituitary gland to increase the release of antidiuretic hormone [37]. Excessive salt intake causes elevated levels of Na^+ which causes vasoconstriction and increases the pumping force and consequently hypertension [38]. The elevated level of Na^+ in the SF group is an indication of the hypertensive effect of high salt load. The increased Na^+ concentration may mean that *Aloe vera* gel stimulated the renin angiotensin aldosterone system or is probably rich in Na^+. Increased Na^+ levels in blood (hypernatraemia) represents deficit of water in relation to the body's sodium stores [39] which may be as a result of impairment of thirst or access to water. The increased Na^+ concentration in the *Aloe* group may also mean that *Aloe vera* gel decreased body water by probably impairing the thirst centers of the hypothalamus and/or increasing urinary excretion of water. Administration of *Aloe vera* gel however reduced the hypertensive effect of high salt intake as Na^+ concentration was significantly decreased in SF+*Aloe* group compared with SF group. Entry of Na^+ into a cell is accompanied by water to increase volume. Despite the elevated levels of Na^+, in all experimental groups, MCV was not increased in these groups but was rather decreased. This may mean that *Aloe vera* gel and salt loading inhibited the Na^+-K^+- ATPase preventing the entry of Na^+ and water into the RBC to increase intracellular volume.

K^+ concentration was significantly increased in SF and SF+*Aloe* groups compared with control and *Aloe* groups. This is in contrast with report of Ofem et al. [36] who reported decreased K^+ concentration following salt loading. The increase in K^+ concentration observed in this study could mean that salt loading decreased renal potassium excretion.

Cl^- concentration was significantly increased in all experimental groups compared with control. It was also significantly increased in SF group compared with *Aloe* group and decreased in SF+*Aloe* group compared with SF group. This result for Cl^- concentration is similar to that of Na^+ in all experimental groups. This increase in the *Aloe* group could also arise as a result of inadequate water intake or loss of thirst

perception as described above. The increase in Cl^- following high salt load is consistent with Ofem et al. [36]. Na^+ and Cl^- move hand in hand. It is obvious that the increase in Cl^- observed in the SF group is because they were fed on NaCl diet. Na^+ reabsorption is coupled with Cl^- reabsorption. The same is applicable to their excretion [40].

HCO_3^- concentration was significantly increased in *Aloe* and SF+*Aloe* groups compared with control but it was not significantly different between control and SF groups. Increase HCO_3^- is an indication of increased metabolic activities. HCO_3^- is a marker for measuring the pH of blood. It acts as a buffer to maintain the pH of blood and body fluids [41]. The present results show that *Aloe vera* gel has the ability to increase blood pH.

CONCLUSION

High salt intake altered red cell indices and posed threat to the immune system of Wistar rats. *Aloe vera* gel was unable to reverse the alterations in red cell indices associated with salt loading but demonstrated an immune-stimulatory effect. Both *Aloe vera* gel and high salt load caused electrolyte imbalance.

AUTHORS' CONTRIBUTION

This research was carried out in collaboration by all authors. ANA conceived and designed the study and performed the data analysis. ALU, AAA and SAL carried out the laboratory work and collected the data. ALU interpreted the results and wrote the first draft of the article. ANA edited the initial draft. All authors read and approved the final manuscript.

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RESEARCH ARTICLE

Isolation of Enterobacteriaceae and non-fermenting Gram-negative bacilli (NFGNB) from Dental Unit Water Lines (DUWL) in a tertiary care institutional setup

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ABSTRACT

Background: The quality of dental unit water lines (DUWL) is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from dental units which thereby influence the individual patient outcome and health-care associated morbidity. The aim of the present study was to determine the microbiological quality of water used, presence of biofilms and also the potential of isolated bacterial species in producing biofilms within DUWL.

Methods: Thirty DUWL samples were collected from various departments of Manipal College of Dental Sciences, Mangalore. Bacteriological analysis was done for the presence of various bacterial contaminants. Presence of biofilms on DUWLs and potential of bacterial isolates to form biofilm were also determined.

Results: Seven of 30 samples (23.3%), were found to be of unsatisfactory quality (coliform count > 200 CFU/ml), most frequently from air/water syringes. A total of 45 strains were isolated from 14 water samples. Genera isolated were *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp. Four of 10 samples from DUWL tubing showed presence of biofilms (40%), formed mostly by *Acinetobacter* spp. and *Pseudomonas* spp. Out of 45 strains that were isolated, 19 strains displayed ability to form biofilms. Maximum number (10) isolates formed biofilms with 48 hours.

Conclusion: Exposure to contaminated water from DUWL poses threat to the well-being of the patient and the health care personnel as well. Hence, measures should be initiated to ensure the optimum quality of DUWL water.

Keywords: Enterobacteriaceae; Non-fermenting Gram-negative bacilli (NFGNB); Dental Unit Water Lines (DUWL).

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INTRODUCTION

Dental unit water lines (DUWL) plastic tubes which deliver water to hand-held instruments that are routinely used in dental procedures. The significance of the quality of these DUWLs cannot be over-emphasised since the health-state of the patient and the dental staff are considerably influenced [1]. These DUWLs are known to be colonised by multiple micro-organisms. Another significant challenge about these microbes is their ability to rapidly form biofilms following colonization [1]. Biofilm formation is an important survival strategy of these organisms that enable prolonged persistence which in turn is associated with multiple health hazards. Consequently, the bacteriological quality of dental unit water lines is usually unacceptable with high coliform count, sometimes as high as >10^6 CFU/ml [2]. Though available evidences suggest that these microbes are non-pathogenic to healthy individuals, these cause considerable morbidity in immuno-compromised patients and those with generalised severe illnesses [3]. Other than immuno-compromised patients, these microbes cause opportunistic infections in pregnant women, transplant-recipients, aged, alcoholics and smokers. Several studies have reported the isolation of various bacteria such as *Streptococci* spp., *Staphylococci* spp., *Pseudomonas* spp., *Legionella, Escherichia* spp. and few other Gram-negative bacilli [4-7]. According to the Centre for Disease Control (CDC), the recommended coliform count of dental water should be < 500 CFU/ml of aerobic heterotrophic bacteria. But the American Dental Association (ADA) has further reduced the standard cut-off coliform count to < 200 CFU/ml of aerobic heterotrophic bacteria [3]. In this study, ADA guidelines were followed to interpret the bacteriological quality. In this study, the bacteriological quality of DUWL is assessed and characterised on the basis of various parameters like isolation rate, isolated genera/ species, presence of biofilms and the ability of isolated bacteria to form biofilms.

MATERIALS AND METHODS

The study was conducted in the department of microbiology, KMC, Mangalore in collaboration with the clinical department of Manipal College of Dental Sciences, Mangalore. The samples were collected from a total of 10 dental units. From each unit, the water samples were samples were obtained from:

- Air/water syringe - 3 in 1 syringe designed to deliver air, water or air/water into mouth during dental treatment.
- Mouth-wash water-water
- Air rotor water sample.

Hence, it is three water samples from each unit making a total of thirty (n=30) samples.

Sampling of DUWL

30 DUWL samples were collected randomly from 10 dental units at MCODS Mangalore. All the units were supplied with containers for the collection of water samples.

Bacteriological analysis of DUWL samples

- 50 ml volumes of samples collected from air/water syringe, mouth-wash water and air rotor after disinfecting the tip with 70% alcohol.
- Then inoculated into multiple tubes of MacConkeys broth (double/single strength).
- Incubated at 37^{O} C for 48 hours.
- Coliform count per 100 ml was estimated from number of tubes showing acid/gas production.

Isolation and identification of bacterial isolates from DUWL samples

The collected water sample was filtered using membrane filters. Then the organisms were washed by vortexing the membrane in a container containing 10 ml of sterile PBS for 1 min. These samples were inoculated into BHI broth for observation of bacterial growth. The sample showing growth after suitable incubation period was processed further for the identification of the isolate by standard microbiological methods [8].

Detection of biofilm formation on the DUWL

External DUWS tubing surface was wiped with a sterile alcohol wipe. The tubing was sectioned to obtain a specimen representing 1 cm^2. The surface was rinsed with sterile PBS to remove planktonic cells. Using sterile dental probes, the surface of the biofilm was scraped into 1 ml of sterile PBS. These biofilm samples were then inoculated into BHI broth to observe bacterial growth. Any sample showing growth was processed further for the identification of isolate by standard microbiological methods [8].

Determination of the capacity of bacterial isolates to form biofilm

Bacterial strains isolated from DUWL samples were used to determine their capacity to form biofilms using microtitre plate method [9]. Aliquots of 200 µl of the standardized test bacterial suspension in Lauria broth was transferred into pre sterilized 96-well polystyrene microtitre plates. Incubate at 37°C for 6 hours. 25 µl of 1% crystal violet added to each well, shaking the plates three times to help the colorant to get the bottom of the well. After 15min at room temperature, each well is washed with 200 µl sterile PBS to remove the planktonic cells. Washing was repeated for 3 times. The adhered bacteria forming biofilm was remained on the surface of the well. Crystal violet bound to the biofilm was extracted later with 2 washings with 200 µl of ethyl alcohol. The alcohol was then transferred into a glass tube containing 1.2 ml of alcohol and agitated. The degree of biofilm formation was determined by spectrophotometer at 540 nm. The obtained data is used to classify strains.

Data analysis

Results obtained were analysed using Microsoft Excel.

RESULTS

In this study, we analysed the bacteriological quality and collectively studied various bacteriological characteristics like isolation of different bacteria, identification of isolates, detection of the presence of biofilms and assessment of the capacity to form biofilms, in a total of thirty (n=30) water samples collected from 10 random dental units. Out of the 30 samples, the presumptive coliform count of seven (n=7, n/N=7/30, 23.3%) samples were found to be higher than the acceptable limits (i.e. > 200 CFU/ml) with reference to the ADA recommendation (Table 1). Out of the seven (n=7) samples with unacceptable bacteriological quality, five (n=5) samples were collected from 3 in 1 air/water syringe and two (n=2) samples were collected from air rotor.

All the thirty (n=30) water samples were passed through membrane filters and were further cultured in BHI broth. Isolation rate was 46.7%. Several genera of bacteria were grown from fourteen (n=14, n/N=14/30, 46.7%) samples. From fourteen (n=14) samples, 45 genera of bacteria were isolated (Table 2). Most of the samples yielded multiple isolates. Out of the 45 isolates obtained from 14 samples, the isolation rates for *Escherichia* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Acinetobacter* spp. were 28.9% (n=13, n/N=13/45), 22.2% (n=10, n/N=10/45), 22.2% (n=10, n/N=10/45), 15.6% (n=7, n/N=7/45) and 11.1%

(n=5, n/N=5/45) respectively. *Escherichia* spp., *Enterobacter* spp. and *Pseudomonas* spp. were the most commonly isolated bacterial genera. Samples collected from air rotor yielded a maximum of twenty one (n=21, n/N=21/45, 46.7%) isolates while those from air/water syringe and mouth-wash water were thirteen (n=13, n/N=13/45, 28.9%) and eleven (n=11, n/N=11/45, 24.4%) isolates respectively.

Table 1. The microbiological quality of collected samples.

Sample	No. of samples with acceptable coliform count	No. of samples with unsatisfactory quality
Air/ water syringe	5	5
Mouth-wash water	10	0
Air rotor	8	2
Total	23	7

Table 2. The spectrum of bacteria isolated from the collected samples.

Sample	*Escherichia spp.*	*Enterobacter spp.*	*Pseudomonas spp.*	*Klebsiella spp.*	*Acinetobacter spp.*	Total
Air/water syringe	4	1	2	3	3	13
Mouth-wash water	3	4	3	0	1	11
Air rotor	6	5	5	4	1	21
Total	13	10	10	7	5	45

A total of ten (n=10) DUWL tubings were collected to detect the presence of biofilms over the surface (Table 3). Out of the collected ten (n=10) tubings, four (n=4, n/N=4/10, 40%) showed the presence of formed biofilms on their surface. Among the four (n=4) detected biofilms, one (n=1) biofilm was formed combinedly by *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp., two (n=2) biofilms were formed by *Pseudomonas* spp. and *Acinetobacter* spp. while one (n=1) was purely formed by *Enterobacter* spp. In total, four (n=4) biofilms yielded eight isolates (n=8).

Table 3. The frequency of biofilms formed by various organisms in the collected DUWL tubing samples.

Sample	*Escherichia spp.*	*Enterobacter spp.*	*Klebsiella spp.*	*Pseudomonas spp.*	*Acinetobacter spp.*
No. of samples	0	1	1	3	3

A total of forty-five (n=45) different strains were isolated from fourteen (n=14) water samples. Out of forty-five (n=45) isolates that were isolated, nineteen (n=19, 42.2%) isolated possessed the ability to form biofilms (Table 4). Three (n=3, n/N=3/13, 23%), three (n=3, n/N=3/10, 30%), seven (n=7, n/N= 7/10, 70%), two (n=2, n/N=2/7, 28.6%) and four (n=4, n/N=4/5, 80%) isolates of *Escherichia* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Acinetobacter* spp. respectively possessed the capability to form biofilms. The potential to form biofilms was observed maximum with *Pseudomonas* spp. (70%) and *Acinetobacter* spp. (80%) isolates. It is noticeable that four (n=4, n/N=4/5, 80%) out of five (n=5) isolates of *Acinetobacter* spp. possessed the ability to form biofilm.

Table 4. Table representing the potential of various isolates obtained from the collected samples to form biofilms.

Sample	Air/ water syringe		Mouth-wash water		Air rotor		Total	
	No. of isolates	No. of isolates with the ability to form biofilms	No. of isolates	No. of isolates with the ability to form biofilms	No. of isolates	No. of isolates with the ability to form biofilms	No. of isolates	No. of isolates with the ability to form biofilms
Escherichia spp.	4	1	3	0	6	2	13	3
Enterobacter spp.	1	1	4	1	5	1	10	3
Pseudomonas spp	2	2	3	2	5	3	10	7
Klebsiella spp.	3	1	0	0	4	1	7	2
Acinetobacter spp.	3	2	1	1	1	1	5	4

Out of nineteen (n=19) isolates that exhibited the ability to form biofilms, four (n=4, n/N=4/19, 21%) isolates formed biofilms within 24 hours, ten (n=10, n/N=10/19, 52.6%) isolates formed biofilms within 48 hours and five (n=5, n/N=5/19, 26.3%) isolates formed biofilms within 72 hours (Table 5). Majority of *Escherichia spp.* isolates (n=2, n/N=2/3, 66.7%) formed biofilm only between 48 to 72 hours while majority of isolates of the other genera formed biofilms within 24 to 48 hours.

Table 5. The ability of various isolates to form biofilms with respect to the duration of incubation.

Sample	24 hrs	48 hrs	72 hrs	Total
Escherichia spp.	0	1	2	3
Enterobacter spp.	1	1	1	3
Pseudomonas spp.	2	4	1	7
Klebsiella spp.	0	1	1	2
Acinetobacter spp.	1	3	0	4
Total	4	10	5	19

DISCUSSION

The current study is a qualitative assessment and characterization of the microbial contamination of DUWL. In the present study, the bacteriological quality of seven samples was unacceptable according to ADA definition [6]. Previous studies have reported a contamination rate of as high as 96% [10]. In the present study, the frequency of contamination was higher in the samples collected from air/water syringe followed by air rotor. Few studies have recorded an inverse frequency [11, 12] while one more study has reported no significant difference [10]. A total of forty-five isolates were obtained from fourteen water samples which signify contamination of water with multiple bacterial genera. *Escherichia* spp. were the commonest isolates followed by *Enterobacter* spp. and *Pseudomonas* spp. Fotedar et al. [7] have recorded the isolation of Coagulase negative *Staphylococci.* Siang et al. [13] have documented the isolation of *Pseudomonas aeruginosa* and *Legionella pneumophila.* The death of an 81-year female patient who contracted Legionnaire's pneumonia from contaminated dental unit water line has been reported in Italy [14]. Another study undertaken by Smith et al. [15] reported the isolation of oral *Streptococci*, *Pseudomonas* spp. and *Staphylococcus aureus.* There is a

great variation in the microbiological quality and the frequency of isolation different organisms in the existing literature. These wide variations can be attributed to the loco-regional variations in the quality of water supplied, the source of water, the variations in the oral microbial flora and the effectiveness of periodic decontamination.

Biofilms were detected over four out of ten tubings that were sampled. Ten isolates were isolated from these biofilms. A maximum frequency was observed with *Pseudomonas* spp. and *Acinetobacter* spp. Owing to the stagnation of water within the tubings, the microbes settle over the inner surface of the tubings that initiates a sequence of physiological alterations resulting in colonization, micro colony formation and eventually biofilm development [16]. Out of the forty-five isolates, nineteen isolates possessed the ability to form biofilms. The maximum ability to form biofilms was observed with *Pseudomonas* spp. and *Acinetobacter* spp. However, isolates belonging to other genera (*Escherichia* spp., *Enterobacter* spp. and *Klebsiella* spp.) also possessed a moderate ability to form biofilms. Ten of the isolates formed biofilms within 24 to 48 hours while four strains formed biofilms within 24 hours. This poses a significant threat since stagnation of water within the tubings for just 24 to 48 hours might result in colonization and biofilm formation that throws a potential risk to patients and dental care workers.

Currently, there is no available evidence that demonstrates a public health issue due to DUWL exposure. However, minimizing the risk of pathogen exposure will ensure a safe working ecosystem both for the health care workers and the patients. Especially, the immunocompromised patients are at a high risk of developing opportunistic infections following exposure to contaminated DUWL. Dental health care workers are also constantly exposed to aerosols from the dental equipment every day. Unsatisfactory quality of DUWL predisposes the dental personnel to the risk of developing respiratory tract infections especially, if colonised by *Legionella pneumophilia*. Hence, it is essential to ensure the optimum microbiological quality of DUWL by periodic surveillance and regular decontamination measures. As per the recent evidences, the usage of continuous water stay systems with chemical action such as IGN EVO Calbenium and Sterispray would be a superior modality [17].

CONCLUSIONS

The dental unit water lines favor rapid development of biofilms on DUWLs, combined with generation of potentially contaminated aerosols. Contaminated water from DUWL might be consumed, inhaled as aerosols or might contaminate operating site. Exposure to water/aerosols containing bacteria (especially nosocomial pathogens with higher intrinsic antimicrobial resistance such as *Pseudomonas* &*Acinetobacter*) in debilitated patients may lead to life-threatening infections. Therefore it is important to not only maintain a supply of good quality water but also to keep regular quality control checks and regular sterilization/disinfection of dental units.

AUTHOR CONTRIBUTIONS

HJN, VSK and SH conceptualized the study. HJN, VSK and EA drafted the protocol for carrying out the study. HJN, VSK and SH collected samples and carried out the bench work. EA and HJN analysed the results and prepared the manuscript. All the authors read the final draft of the manuscript and approved.

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REVIEW ARTICLE

Cucurbitaceae - the family that nourishes and heals

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ABSTRACT

A review of the activities of members of the Cucurbitaceae was carried out. Many of them are confirmed nutritious and therapeutical. Their global spread, diverse genera and phytochemical profile further confirm them as an attraction for the growth and survival of humanity. The need for alternative control measures to address resistance has heightened the passion for Cucurbitaceae in bioprospecting.

Keywords: Cucurbitaceae; Therapeutic; Nutritional; Extraction; Bioprospecting.

INTRODUCTION

The Cucurbitaceae is known to be the largest family of vegetable crops [1]. The origin of Cucurbitaceae is tropical, and most of its genera originated from Asia, America and largely Africa. Generally, referred to as cucurbits, they are categorize mainly into Zanonioideae and Cucurbitoidea. The subfamily Cucurbitoideae is mainly food plants and particularly vegetables [2] having all the essential constituents required for good human health [3, 4]. The number of the genera is still mired in controversy. Purseglove [5] reported that the family Cucurbitaceae comprised 9 genera and 15 species of vegetables and fruits with different varieties being cultivated throughout the world while Chakravarthy [6] reported 117 genera and 825 species and Yamaguchi [7] confirmed 100 genera and more than 750 species. Also, Subrahmanyam [8] reported 100 genera and 800 species along with Mabberley [9] who indicated 122 genera and 940 species that are distributed in tropical and warm temperate regions of the world. Despite these disagreements, the cucurbitaceae has been an interesting and an outstanding family of dicotyledons, distributed widely over the tropical parts of the world [10].

NUTRITIVE IMPORTANCE

These Cucurbitaceae are known also for their nutritive and medicinal values. For example, *Cucumis sativa* are in treating indigestion and constipation [11]. Cucumber is a widely cultivated plant of gourd family which is eaten in the unripe, green form. The aqueous extract of fruits from same plants revealed the presence of glycosides, steroids, flavonoids, carbohydrates and tannins. High water content and they contain vitamin A and C, flavone glycosides such as isovitexin, saponarin and various acylated flavones [11, 12]. The important genera belonging to the family are *Trichosanthes, Lagenaria, Luffa, Benincasa, Momordica, Cucumis,*

Citrullus, Cucurbita, Bryonopsis and *Corallocarpus*. Some species that have attracted scientific investigation include Momordica *charantia, Cucurbita pepo, Cucumis sativus, Cucumis melo, Citrullus colocynthis, Luffa echinata, Trichosanthes kirilowii, Lagenaria siceraria, Beninca sahispida* [13]. Water melon (*Citrullus lanatus*) contains almost 95% water, small amounts of protein, fat, minerals and vitamins. The major nutritional components of the fruits are carbohydrates, vitamin A and lycopene. Lycopene content of the new dark red watermelon is higher than in tomato, pink grapefruit or guava. Lycopene is a red pigment responsible for watermelon flesh colour, which is an anticancer agent. High amount of water content of watermelon makes it a powerful diuretic diet [14]. The watermelon fruit is widely consumed and rich in water and pectin. Pectin is a substance used in jams for thickening and is believed to offer protection from radiation. It is also traditionally used to treat cardiovascular disease and kidney problems, fever, pain, and inflammation [15].

MEDICINAL USES

Cucurbit plants were used actively as traditional herbal remedies for various diseases. They have demonstrated anti-inflammatory, antitumor, hepatoprotective, cardiovascular andimmune-regulatory activities [13, 16]. Members of this family have always been considered as a subject of research due to the fact that they have a lot of biological activities like anti-fungal, anti-bacterial, anti-viral, anti-diabetic, anti-tumor and anti-AIDS [17] from Cuba through the gulf of Mannar [18] to Nigeria. Ethanolic extract of leaves and stems of *Cucumis sativus* possessed many phytoconstituents such as alkaloid, glycoside, steroid, saponin and tannin except gum, flavonoid and reducing sugars. Its fruit extract has shown free radical scavenging and analgesic activities in mice. The seeds can be used to expel parasitic worms. The juice from the leaves induce vomiting and aid digestion [15].

Trichosanthes cucumerina also called snake gourd, is mainly consumed as a vegetable being rich in protein and vitamin C, carbohydrate, fibre, iron, phosphorus, vitamin B1, vitamin B2 and niacin [19]. The major active constituents of the drug are triterpenoids, saponins, cucurbitacins. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active [20]. Anti-inflammatory activity is exhibited by the root tubers and antidiabetic activity by the seeds. Both the root and fruit are considered to be cathartic. It is used in the treatment of bronchitis, headache, fever, abdominal tumors and skin allergy. Seeds have antibacterial, anti-spasmodic, insecticidal and gastro protective properties. *Momordica charantia* has been used in various Asian traditional medicine systems for a long time, originally for non-communicable diseases like asthma, burning sensation, constipation, colic, diabetes, fever (malaria), gout, helminthiases, inflammation, and ulcer. It has also been publicized to have hypoglycaemic (antidiabetic) properties in animal as well as human studies. The juice of *Momordica charantia* were leaves used to treat piles totally, treating and preventing liver damage, menstrual troubles, burning sensations, constipation and blood purification due to its bitter tonic properties damage. Also, the leaves of *Momordica charantia* are used in treatment of menstrual troubles, burning sensation, constipation, fever (malaria), worms and parasites.

ANTI-WORMS AND ANTI-PARASITIC ACTIVITY

Worms and helminthiases are treated with infusions from *Momordica charantia*, *Cucumis sativa*, and *Praecitrullus fistulosus* capsules and tinctures are widely available in the United States for the treatment of many morbidities. In India, *Momordica charantia* used by tribal people for abortions, and as anthelmintic [21, 22]. The snake gourd called *Trichosanthes cucumerina* is very common in Srilanka and India. All parts of the plant have their medicinal value. The root of the plant has been used for curing boils, headaches, bronchitis. The fruit and seeds are used for anthelmintic and stomach disorder respectively. Kar et al. [23] found out also that the root extract has anti-inflammatory activity while the seed has antidiabetic activity. The antihelminthic potentials of the family were revealed with the experiment on *Lagenaria siceraria* Mol having a cidal effect on earthworm and tapeworm [24]. Earlier in 1987, Elisha et al. investigated the action of *Cucurbita maxima*,

Cucumis sativus and *Lagenaria siceraria* on *Hymenolepis nana* (tapeworm) and *Aspicularis tetraptera* (pinworm) infections in mice in Iraq. The seed extracts of these plants were very effective in controlling the helminthes

ANTIFUNGAL ACTIVITY

The leaves and stem extract of *Cucumis sativus* were investigated for the antifungal potential and found to be effective on *Aspergillus niger* with the dermal mycotic fungus *Microsporum* sp having the least sensitivity [25]. *Momordica charantia* was effective tool in antifungal activity [26]. Many notorious plant pathogens like *Fusarium* have been controlled by the *Momordica* seed extract (MSE) making it a sustainable alternative to synthetic fungicide [27].

Sometimes, the solvent effect was noted in the efficacy of extracts of Cucurbitaceae on fungi. The n-hexane and ethyl acetate extract of *M. charantia* were effective against *Saccharomyces cerevisiae* while chloroform and ethyl acetate produced the best sensitivity against *Candida albicans* [28]. The antifungal activity assay four phytopathogenic fungal species included *Pythium aphanidermatum, Botryosphaeria dothidea, Fusarium oxysporum f.sp. cucumerinum* and *Botrytis cinerea* was performed on the stem of *Cucumis sativus*. Results revealed an appreciable sensitivity arising from the ethanol fractions of the sphingolipids derived from the plant [29].

Lagenaria siceraria called Bottle Gourd is a vegetable which is commonly consumed in India. It is a good tonic especially for the heart [30]. It is effective against diseases such as fever, pectoral cough, bronchia disorders, ulcers and pain [31]. It also has diuretic activity [32]. Acetone has high throughput when the matrix is *Lagenaria siceraria.* Another species of therapeutic importance is *Wibrandia ebracteata* which has been effective for traditional medicines. Studies have shown that both tuber and roots have analgesic and antitumor properties. It also has anti-inflammatory and arthritis properties [33].

ANTIBACTERIAL ACTIVITY

The increasing rate of resistance to common antibiotics among bacterial and fungal species calls for a concerted effort at combating communicable diseases in animals and man. Antibacterial effects have been investigated using ethanolic extract of some leaves. The result showed that *Bacillus subtilis* and *E. coli* were sensitive to the intervention [34].

Bacterial-mediated infections are controlled by many species in the family using local infusions and concoctions or by laboratory activities involving other solvent extractions antimicrobial activities of petroleum ether, chloroform, ethyl acetate and methanol extract of the leaves of *Momordica charantia* were effective against various pathogenic bacteria such as *Bacillus cereus*, *Enterobacter faecalis*, *Salmonella paratyphi*, *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial potency of this plant extract is due to the presence of phenolic compounds flavonoids and carotenoids [19]. *Momordica charantia* root is used in the treatment of syphilis, rheumatism, ulcer, boils, and septic swellings. In the gulf of Manna, Gurudeeban et al. [35] confirmed the efficacy of *Citrullus colocynthis* on some bacterial species. The wax gourd, also called *Benincasa hispida* has useful seeds, peel and pulp particularly in inhibiting disease causing microbes. The interest in the antimicrobial activity of *B. hispida* was ignited by the success recorded on *Serratia spp., Shigella boydii, Pseudomonas aeruginosa* and *Salmonella typhimurium* [36]. The plant also has the potential to serve as preservative. *Benincasa hispida* also called wax gourd is a known vegetable in most of the tropical countries that has a high medicinal value. Particularly in curing internal bleeding, epilepsy, cough, asthma, diabetes and nervous disorders [37].

Spectacular performance was recorded *Momordica charantia*, bitter melon, on many bacterial species most of which are plant and human pathogens [38].

In Nigeria, a lot of the members of this family have been exploited locally to treat infectious diseases. Osuagwu and Ejikeme [39] had reported the inhibition of growth of *Salmonella typhi*, *Enterococcus faecalis*

and *Pseudomonas aeruginosa* from extracts of *Trichosathes cucumerina*. Aqueous extracts were less effective than ethanolic fractions on the tested microbes*Trichosanthes tricuspidata* has been used as a laxative and for migrane treatment. Its root extract has anti-oxidant properties [40]. Generally, bacteria were more sensitive to the extracts from this *M. charantia*, than fungi just as Gram negative bacteria were more susceptible than the Gram positive strains [41].

Cucurbita pepo popularly called pumpkin is an example of medicinal cucurbits like the other cucurbits; ripe fruit of *Cucurmis pepo* are eaten in many parts of the world where it is cultivated. Pumpkin seeds a moderate to very good source of a variety of nutrients, including minerals, protein and healthy fats [42]. They are good source of important minerals such as copper, zinc, iron and magnesium. Also, the seeds are used in nutrition in various forms. The seeds can be eaten raw, and they are good sources of lipids and proteins [43]. Seeds oil of pumpkins is a good raw material for the production of oil used in preparation of food and also for medicinal use [44]. Cultivation of pumpkin is not only for food only but also for their medicinal properties. Medicinal properties particularly have been attributed to all the part of the fruit and the plant. Pumpkin seed aids with appetite stimulation, and it's beneficial for the teeth, nerves, hair and nails [45]. It is a climbing herb and cultured mostly in America and tropical India. It is used for increasing appetite, blood purification, and leprosy. It also helps in proper management of benign prostatic hyperplasia which is heavily related to prostate cancer [46, 47].

Cucumis melo also called musk melon has been linked to the cure for chronic eczema. It is diuretic, diaphoteric, laxative, good tonic and has anti inflammatory properties. The bitter apple whose botanical name is *Citrullus colocynthis* is grown in arid places and good for hypoglycemia, tumors, ulcers, asthma, bronchitis, constipation; it's an analgesic and has anti-inflammatory activities [48, 49, 50].

In 2017, the antimicrobial activity of *Cucurbita moschota* and *Lagenaria siceraria* was investigated by Dash and Gosh [51] on *Acinetobacter baumanii*. The lethal concentration LC_{50} of *C. moschata* and *L. siceraria* was 70 and 135 respectively. The candidate control agent was their seed protein hydrolysate making it both of high nutritional and antimicrobial importance in the food security and safety system. Using the disc diffusion method on some bacteria, Hansanuzzanan et al. [17] carried out preliminary evaluation of the root extract of *Coccinia grandis* on *Staphylococcus aureus*, *Bacillus cereus* and *E. coli*. In comparison with standard ciprofloxacin, the ethanolic extracts resulted in 9-12 mm zone of inhibition with an equally significant cytotoxicity at LC_{50} of 2.49 mg/ml. Of all the solvents tested - carbon tetrachloride, n-hexane, water and dichloroethane, the latter was most effective.

ANTIVIRAL ACTIVITY

The protease inhibitors were reported to incite a significant antimicrobial action [52]. Extracts from the leaves of *Momordica charantia* was also effective against hepatitis. Some ribosome inactivating protein RIPs were once isolated from *Momordica charantia* opening ways for antiviral therapy [53]. Puri et al. [53] had investigated the mechanism of action of ribosome inactivating protein (RIPs) of M charantia and concluded that the RIPs are not only very effective against both HBV and HIV, it is also non- toxic to host's normal cell. The RIPs have a future in anti-cancer and anti-viral therapy as they target specifically, hosts protein synthesis. The expression of hypoglycaemic peptide MC6 from *M. charantia* in *E. coli* was investigated by Wang et al. [54]. The research revealed the ease of modification of the bacterial biochemistry by this plant, a characteristic that can exploited to achieve both in vivo and in vitro pathogenic therapy. In *C. balsamina*, substrate specificity is a common property binding all members of cucurbitaceae. Also peptide bioavailability can be determined by the distribution of the molecular weight of peptides in seed protein hydrolysates which are the main sources of bioactive peptides [54].

Many extracts have comparable antimicrobial profile as the standards antibiotics. For example, piperacillin (100 µg/disc) and gentamicin (10 µg/disc) recorded similar sensitivity pattern when some bacterial and fungal strains were challenged with the leaf extracts of *Citrullus colocynthis*. The combined effects of the

flavonoids, phenols and tannins present had significant antimicrobial activity, a situation that invites its possible use in plant disease control [55].

Telfaria occidentalis common in Nigeria has the ability to be hepato-protective and antimicrobial. Some of the attributes of this plant have been linked to its antioxidant value [56].

Luffa cylindrica another Cucurbitaceae very common in Nigeria, was investigated for the properties of its seed oil. It was discovered that the seed oil exhibited significant antimicrobial activity against *S. aureus*, *Candida albicans* and *E. coli*. The report also confirmed that the efficacy of the extract was on par with that of afloxacin, the synthetic antibiotic [57].

Luffa echinata which is also known as bundal has been recommended for treating liver related diseases due to the presence of saponins, echinatol A and B which are active for curing such disease [58]. Some of the Curcubitaceae are used as an anti-inflammatory agent and expectorant. An example of such is Chinese cucumber which has also been reported to be potential anti-tumor promoters. [59]. *Cucurbita ficififolia* has been used in the preparation of dishes for eating and also for candies preparation. It can also be used for curing wounds and ailments like fever and hemorrhoids. It is also linked to be a good drug for treating diabetes type 2 [60].

The extracts of acetone water and ethanol were very effective against uropathogenic *E. coli* being particularly effective against the extended spectrum of beta lactamase (ESBL) producing species [61].

The window of members of this family was further opened by the discovery of the phytochemicals from *Icacina trichantha* and the antimicrobial activity of the extract. The potentially therapeutic significance of the bioactive compounds in this leaf extract was proven when cells of *Klebsiella* and *Candida* were controlled when challenged [62].

ANALYTE EFFICACY IN RELATION TO METHOD OF EXTRACTION

Solvent efficacy plays a vital role in extracting the phytochemicals. As demonstrated by Fidrianny et al. [63] on the leaves of *Luffa acutangula, Sechium ledule,* and *Momordica charantia*, polarity of solvents can dictate the ease of recovery. There were differences in extraction using n hexane, ethanol and ethyl acetate. This is a factor to be considered in determining the choice of plant for antimicrobials and antioxidant [64]. The mode of determination of active ingredients also matters. For example, the total flavonoid methods were used with success on *Momordica charantia* [65].

Using the GC MS method, the major components of *Cucurbita pepo* were 9,12-octadecadienoic acid (47.17%) [66]. When the bark of *Cucumis* was retained it had a better antibacterial effect than when removed. This suggests that the bark is concentrated with the desired analytes for antibiosis [67]. It was also shown that different organs of the plant have different concentration levels of various. The total polyphenol contents of squirting cucumber (*Ecballium elaterium*) differed remarkably in all the organs tested [68]. With precision analyses, many antifungal products have been extracted from many plants in the family [69]. Using different polarity extracts, that employed DPPH (2.2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) fractions, and correlation of total phenolic, flavonoid and carotenoid content the antioxidant capacities were measured using the leaves. It was discovered that the ethanolic extract of *Luffa acutangula* leaves had the highest DPPH scavenging capacity ethyl acetate extract of *Sechium edule* leaves had the highest FRAP capacity; n-hexane extract of *Momordica charantia* leaves had highest flavonoid and carotenoid contents [63]. The implication of this is that there always a correlation between the profiles of ingredients extracted and the polarity of the solvent used. Using affinity chromatography on alpha agarose lactose matrix, lectin was isolated from *M. balsamina* seeds with high throughput resembling the yield from other members of the family [70]. Quercetin, a flavonoid and an important therapeutic component of the leaf, stem and root of *Citrullus colocynthis* was successfully purified through series of solvents involving chloroform, ethyl acetate diethyl ether and run through a TLC and subsequently HPLC process [71]. The pressurized boiling system through gas Chromatography mass spectrometry gave a high yield of extracts from *Momordica charantia* [72]. This method was more effective than the conventional Soxhlet method. Amino acid

profile and the antimicrobial content are co related when studied on *Cucurbita moschata* and *Lagenaria siceraria* [51] suggesting one serving as an indicator to the other.

THE FUTURE OF THERAPY WITH CUCURBITS

The increasing rate of resistance to common antibiotics among bacterial and fungal species calls for a concerted effort at combating communicable diseases in animals and man. With the WHO raising alarm on the increasing level of resistance among bacterial strains, bioprospecting into the world of cucurbits becomes an attraction. Many of the developing worlds recognise the need for phytotherapy [73, 74] however, the original native interventions involving concoctions and infusions of this group invites better embrace. With the embrace come refining and developing better products regarding mode of extraction, toxicity studies and overall efficacy. With some members identified and confirmed as toxic to mice [75] issues bordering on concentration purity, species of mammal involved and stability at administration make good research directions.

With a report stating that many Nigerian plants, including all Cucurbitaceae are disappearing fast [76], it has become necessary for governments in Nigeria and other African countries to recognize the need for vigilance towards traditional medicine. Even when molecular analyses expose the possibility of new species and apparent relationship with others [77], conscious efforts at replacing and massive tree planting is a critical response to the present scare

It is noted that phytoremediation is carried out using members of the Cucurbitaceae. For example, they can to up take organochlorines. Seven cultivars of the *Lagenaria siceraria* species were used to determine their capacity to remediate heptachlor- and heptachlor-epoxide-contaminated soil. The seven *Lagenaria* cultivars were grown in contaminated and uncontaminated soil for 13 weeks. The results showed that all the plants tolerated heptachlor and heptachlor epoxide in the soil and were able to bear a limited number of immature fruits during the short study period. All seven *Lagenaria* cultivars showed some ability to up take heptachlor epoxide into their vines with bioaccumulation factors [78]. The molecular approach to the ultrastructure of *Momordica* sp. opened the window of the possibility of igniting modifications in the cells *E. coli* [54].

CONCLUSION

In spite of the difficulties recorded in purification and administration of crude extracts of cucurbits for human and animal use, the family still remains a rich and reliable therapeutic resource particularly in the developing countries and therefore, a family of hope and promise.

AUTHOR CONTRIBUTIONS

This research work is a product of agreement and collaboration among all authors. SOF suggested the work and designed it. Extensive literature research was done by SOF, DOJ and AAA. All authors were involved in the writing, review and approval of the manuscript. All authors read and approved the final manuscript.

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RESEARCH ARTICLE

Effect of age on the Br, Fe, Rb, Sr, and Zn concentrations in human prostatic fluid investigated by energy-dispersive X-ray fluorescent microanalysis

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ABSTRACT

The effect of age on Br, Fe, Rb, Sr, and Zn concentrations in human prostatic fluid was investigated by ^{109}Cd radionuclide-induced energy dispersive X-ray fluorescent microanalysis. Specimens of expressed prostatic fluid were obtained from 51 men (mean age 51 years, range 18-82 years) with apparently normal prostates using standard rectal massage procedure. Mean values (M ± SEM) for concentration of trace elements (mg·l^{-1}) in human prostate fluid were: Br 3.58±0.59, Fe 9.04±1.21, Rb 1.10±0.08, Sr 1.08±0.17, and Zn 573±35. An age-related increase in Zn content and decrease in Br and Fe concentration was observed.

Keywords: Human prostatic fluid; Trace element variations with age; Energy dispersive X-ray fluorescent analysis.

INTRODUCTION

The prostate gland may be a source of many health problems in men past middle age, the most common being benign prostatic hypertrophy (BPH), and prostatic carcinoma (PCa). BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra. PCa is the most prevalent male cancer in many populations, including the United States, West European states, Australia, New Zealand, and others [1]. PCa ranks second in incidence and the fifth in mortality in men worldwide [2]. Although the etiology of BPH and PCa is unknown, several risk factors, including age and diet (Zn, Ca and some other micronutrients), have been well identified [3-7]. It is also reported that the risk of having PCa drastically increase with age, being three orders of magnitude higher for the age group 40-79 years than for those younger than 39 years [3, 8].

Chemical elements have essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of chemical elements depend on tissue-specific need or tolerance, respectively [9]. Excessive accumulation or an imbalance of the chemical elements may disturb the cell functions and may result in cellular degeneration or death [9-11].

High intracellular Zn and Ca concentration is probably one of the main factors acting in both initiation and promotion stages of prostate carcinogenesis [12-17]. A significant tendency of age-related increase in Zn and many other chemical element mass fractions in the normal prostate was recently demonstrated by us [18-32]. Moreover, it was found an androgen dependence of some prostatic chemical elements, including Zn [33-44]. Thus, it seems fair to suppose that besides Zn, many other chemical elements, which the prostatic tissue contents increase with age, also play a role in the pathophysiology of the prostate.

According to Deering et al. [45], the prostatic parenchyma contains three main components: glandular tissue, prostatic fluid, and fibromuscular tissue or stroma. Glandular tissue includes 25-30 small glandular units (acini) located in the periphery of the prostate. Prostatic fluid fills the lumina of the acini (glandular lumen). Epithelial cells surround the periphery of the acini and the luminal surfaces in the acini.

It is known that several of prostatic fluid components, such as Ca, K, Mg, and Zn, are at much higher concentrations than the blood serum [12]. Prostatic fluid is secreted by prostatic epithelial cells therefore the composition of the fluid should reflect metabolic activities of the secreting cells. Thus, one might expect that an age-related increase in the prostatic chemical element contents might be observed when the chemical element composition of prostatic fluid is studied. To confirm or refute these hypotheses it is necessary to investigate the age-related dynamics of Zn and other chemical elements in prostatic fluid. At our knowledge there are no studies regarding the effect of age on content of chemical elements in prostatic fluid with the exception of Zn.

The primary purpose of this study was to determine reliable values for the trace element concentrations in the intact prostatic fluids of apparently healthy subjects ranging from young adult males to elderly persons using energy dispersive X-ray fluorescent microanalysis (EDXRF). The second aim was to compare the obtained results with reported data for trace elements in prostatic fluid, as well as with data for trace elements in some fluids of Reference Man. The third aim was to compare the trace element concentrations in prostatic fluid samples of age group 2 (aged 41 to 82 years), with those of group 1 (aged 18 to 40 years) and to check the correlations between age and trace element concentrations in prostatic fluid. The final aim was to estimate the correlations between trace elements' concentrations in intact prostatic fluids of males in two periods of adult life.

All studies were approved by the Ethical Committee of the Medical Radiological Research Centre. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

MATERIALS AND METHODS

Samples

Specimens of expressed prostatic fluid were obtained from 51 men (mean age 51 years, range 18-82 years) with apparently normal prostates by qualified urologist in the Urological Department of the Medical Radiological Research Centre using standard rectal massage procedure. Subjects were asked to abstain from sexual intercourse for 3 days preceding the procedure. The cytological and bacteriological investigations were used to control the norm conformity of prostatic fluid samples chosen for EDXRF.

Sample preparation

Specimens of expressed prostatic fluid were obtained in sterile containers which were appropriately labeled. Twenty μl (microliters) of fluid were taken by micropipette from every specimen for trace element analysis, while the rest of the fluid was used for cytological and bacteriological investigations. The chosen 20 μl of the expressed prostatic fluid was dropped on 11.3 mm diameter disk made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in an exsiccator at room temperature.Then the dried sample was covered with 4 μm Dacron film and centrally pulled on to a Plexiglas cylindrical frame.

Instrumentation and method

The facility for radionuclide-induced energy dispersive X-ray fluorescence included an annular ^{109}Cd source with an activity of 2.56 GBq, Si(Li) detector and portable multi-channel analyzer combined with a PC. Its resolution was 270 eV at the 6.4 keV line. The facility functioned as follows. Photons with a 22.1 keV ^{109}Cd energy are sent to the surface of a specimen analyzed inducing the fluorescence K_α X-rays of trace elements. The fluorescence irradiation got the detector through a 10 mm diameter to be recorded.

The duration of the Zn concentration measurement was 10 min. The duration of the Zn concentration measurement together with Br, Fe, Rb, and Sr was 60 min. The intensity of K_α-line of Br, Fe, Rb, Sr, and Zn for samples and standards was estimated on calculation basis of the total area of the corresponding photopeak in the spectra. The trace element concentration was calculated by the relative way of comparing between intensities of K_α-lines for samples and standards.To determine concentration of the elements by comparison with a known standard, aliquots of solutions of commercial, chemically pure compounds were used for a device calibration [46]. The standard samples for calibration were prepared in the same way as the samples of prostatic fluid. Details of the analytical method and procedures used here for sample preparation were presented in our earlier publications concerning the chemical elements of human prostatic fluid [47, 48].

Certified Reference Material

Because there were no available liquid Certified Reference Material (CRM) ten sub-samples of the powdery CRM IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results.

Computer programs and statistic

Using the Microsoft Office Excel software to provide a summary of statistical results, the arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for all the trace element concentrations obtained. The difference in the results between two age groups was evaluated by parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test. Values of $p<0.05$ were considered to be statistically significant. For the estimation of the Pearson correlation coefficient between age and trace element concentration, between different pairs of the trace element concentrations in the two age groups, as well as for the construction of "individual data sets for trace element concentrations versus age" diagrams the Microsoft Office Excel software was also used.

RESULTS

Table 1 depicts our data for Br, Fe, Rb, Sr, and Zn mass fractions in ten sub-samples of CRM IAEA H-4 (animal muscle) certified reference material and the certified values of this material. Of 4 (Br, Fe, Rb, and Zn) trace elements with certified values for the CRM IAEA H-4 (animal muscle) we determined contents of all certified elements (Table 1). Mean values (M±SD) for Br, Fe, Rb, and Zn were in the range of 95% confidence interval. Good agreement of the trace element contents analyzed by ^{109}Cd radionuclide-induced EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicate an acceptable accuracy of the results obtained in the study of the prostatic fluid presented in Tables 2-7.

Table 2 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Br, Fe, Rb, Sr, and Zn concentrations in prostatic fluid of apparently healthy men.

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn concentrations in the normal human prostatic fluid [49-51] is shown in Table 3.

The differences between the mean of Br, Fe, Rb, Sr, and Zn concentrations in the prostatic fluid and those in blood serum, urine, and breast milk of Reference Man [52] are presented in Table 4.

Table 1. EDXRF data of Br, Fe, Rb, Sr, and Zn contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg·kg^{-1}, dry mass basis).

Element	Certified values			This work results
	Mean	95% confidence interval	Type	Mean±SD
Fe	49	47 - 51	C	48±9
Zn	86	83 - 90	C	90±5
Br	4.1	3.5 - 4.7	C	5.0±1.2
Rb	18	17 - 20	C	22±4
Sr	0.1	-	N	<1

Mean - arithmetical mean, SD - standard deviation, C- certified values, N - non-certified values.

Table 2. Some basic statistical parameters of Fe, Zn, Br, Rb, and Sr concentration (mg/l) in human prostatic fluid.

Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Br	3.62	3.26	0.58	0.49	10.0	1.63	0.498	9.16
Fe	9.04	7.28	1.21	1.27	39.8	7.84	1.29	21.3
Rb	1.10	0.51	0.08	0.38	2.45	1.03	0.41	2.36
Sr	1.19	0.79	0.14	0,036	3.44	1.18	0,037	3.16
Zn	573	202	28	253	948	552	260	941

M - arithmetic mean, SD - standard deviation, SEM - standard error of mean, Min - minimum value, Max - maximum value, Per. 0.025 - percentile with 0.025 level, Per. 0.975 - percentile with 0.975 level, DL - detection limit.

Table 3. Median, minimum and maximum value of means of Fe, Zn, Br, Rb, and Sr concentration (mg/l) in human prostatic fluid according to data from the literature.

Element	Published data [Reference]			This work results
	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M±SD, (n)**	M±SD
Br	-	-	-	3.62±3.26
Fe	-	-	-	9.04±7.28
Rb	2.26 (1)	1.11±0.57 (15) [49]	2.35±1.85 (11) [49]	1.10±0.51
Sr	-	-	-	1.19±0.79
Zn	453 (19)	47.1(-) [50]	9870±10130 (11) [51]	573±202

M - arithmetic mean, SD - standard deviation,(n)* - number of all references, (n)** - number of samples.

Table 4. The differences between the mean of Fe, Zn, Br, Rb, and Sr concentration in the prostatic fluid and in blood serum, urine, and milk of Reference Man (mg/l).

Element	This work	Reference Man [52]			Ratios (t-test)		
	Prostatic fluid	Blood serum	Urine	Breast milk			
	I	II	III	IV	I / II	I / III	I / IV
Br	3.6	4.5	3	1.5	0.80	1.2	2.4
Fe	9.0	1.0	0.075	0.45	9.0	120	20
Rb	1.1	0.2	1.3	0.75	5.5	0.85	1.5
Sr	1.2	-	-	-	-	-	-
Zn	573	0.95	0.25	1.5	603	2292	382

To estimate the effect of age on the concentrations in the prostatic fluid we examined two age groups: group 1 (aged 18 to 40 years, mean=27.5 years) and group 2 (aged 41 to 82 years, M=59.1 years) (Table 5),

calculated correlation coefficient between age and trace element concentration (Table 6), and constructed "individual data sets for trace element concentrations versus age" diagrams with lines of trend (Figs. 1).

Table 5. Effect of age on mean values (M±SEM) of Br, Fe, Rb, Sr, and Zn concentration (mg/l) in human prostatic fluid.

Element	Age groups				Ratios
	Group I 18-40 year (M=27.5) n=13	**Group II 41-82 year (M=59.1) n=38**	**Student's t-test $p\leq$**	***U*-test* p**	**Group II to group I**
Br	6.35±1.17	2.86±0.59	**0.025**	**<0.01**	0.450
Fe	12.1±1.9	8.29±1.42	0.127	>0.05	0.685
Rb	0.91±0.15	1.16±0.10	0.195	>0.05	1.27
Sr	0.87±0.21	1.27±0.17	0.161	>0.05	1.46
Zn	501±47	598±34	0.108	>0.05	1.19

M - arithmetic mean, SEM - standard error of mean, *Wilcoxon-Mann-Whitney *U*-test, **bold -** significant difference ($p\leq0.05$).

Table 6. Correlations between age and trace element mass fractions in human prostatic fluid (r - coefficient of correlation).

Element	Br	Fe	Rb	Sr	Zn
Age	-0.700[c]	-0.420[b]	0.022	0.168	0.292[a]

Statistically significant values: [a]$p\leq0.05$, [b]$p\leq0.01$, [c]$p\leq0.001$.

Table 7. Intercorrelations of the Fe, Zn, Br, Rb, and Sr concentration in human prostatic fluid (*r* - coefficient of correlation).

Age group	Element	Br	Fe	Rb	Sr	Zn
Group 1 18-40 years n=13	Br	**1.0**	-0.315	-0.490	0.696[a]	0.168
	Fe	-0.315	**1.0**	0.753[a]	0.987[b]	0.386
	Rb	-0.490	0.753[a]	**1.0**	0.445	0.309
	Sr	0.696[a]	0.987[b]	0.445	**1.0**	0.832[a]
	Zn	0.168	0.386	0.309	0.832[a]	**1.0**
Group 2 41-82 years n=38	Br	**1.0**	0.714[b]	0.178	0.172	-0.535[b]
	Fe	0.714[b]	**1.0**	0.148	0.410	-0.241
	Rb	0.178	0.148	**1.0**	-0.131	-0.097
	Sr	0.172	0.410	-0.131	**1.0**	0.069
	Zn	-0.535[b]	-0.241	-0.097	0.069	**1.0**
Group 1 and 2 (combined) 18-82 years n=51	Br	**1.0**	0.567[b]	-0.054	0.161	-0.428[a]
	Fe	0.567[b]	**1.0**	0.176	0.392[a]	-0.217
	Rb	-0.054	0.176	**1.0**	-0.111	-0.011
	Sr	0.161	0.392[a]	-0.111	**1.0**	0.186
	Zn	-0.428[a]	-0.217	-0.011	0.186	**1.0**

Statistically significant values: [a] - $p\leq0.05$, [b] - $p\leq0.01$.

For normal physiology of prostate gland it is very important not only absolute values of trace element concentrations in the prostatic fluid, but also their relationships, Therefore, the Pearson correlation coefficientsbetween different pairs of the trace element concentrations in the two age groups separately and combined were calculated and presented in Table 7.

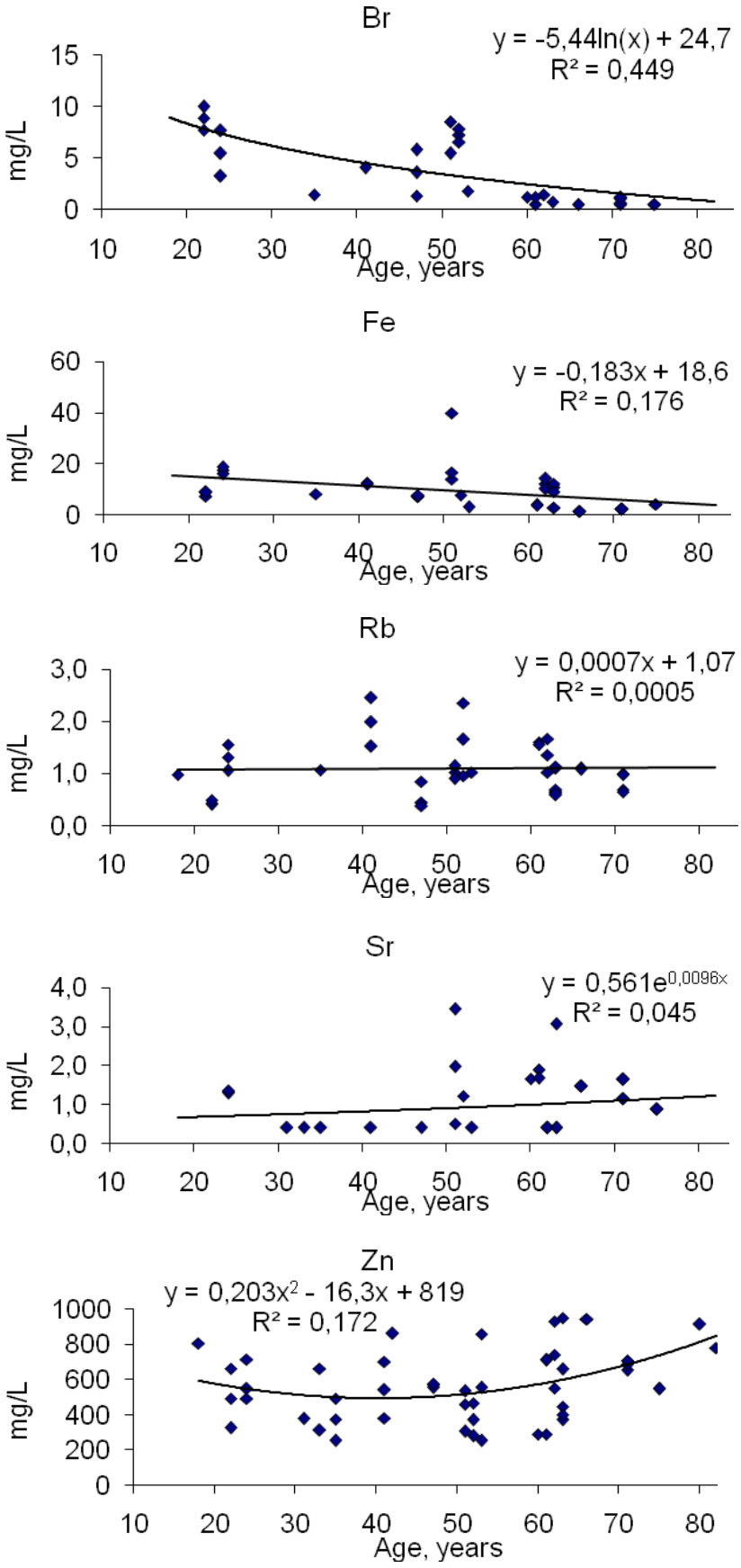

Figure 1. Data sets of individual concentrations of Br, Fe, Rb, Sr, and Zn in prostatic fluid of healthy men and trend of concentrations with age.

DISCUSSION

The mean values and all selected statistical parameters were calculated for 5 (Br, Fe, Rb, Sr, and Zn) chemical elements (Table 2). The concentrations of these elements were measured in all or a major portion of prostatic fluid samples.

The mean of Zn concentration obtained for prostatic fluid, as shown in Table 3, agrees well with median of means cited by other researches [50, 51]. The mean of Rb concentration obtained for prostatic fluid agrees well with our data reported 37 years ago [49]. No published data referring to Fe, Br, and Sr concentrations in prostatic fluid were found.

The obtained mean for Zn concentration in human prostatic fluidis two orders of magnitude higher than mean values of the element content in blood serum and breast milk, and three orders of magnitude higher than in urine (Table 4). The obtained mean for Fe concentration in human prostatic fluidis nearly one order of magnitude higher than that in blood serum and breast milk, and two orders of magnitude higher than in urine (Table 4).The mean for Rb concentration in human prostatic fluidis 5.5 times higher than that in blood serum, and almost equals the mean values of the element content in urine and breast milk (Table 4). So, the human prostatic secretion is a target fluid of human body not only for Zn, but also for Fe and Rb.

A statistically significant age-related decrease in Br concentration was observed in prostatic fluid when two age groups were compared (Table 5). In second group of males with mean age 59.1 years the mean of Br concentration in prostatic fluid was 2.2 times lower than in prostatic fluid of the first age group (mean age 27.5 years). A statistically significant decrease in Br concentration was confirmed by the negative Pearson's coefficient of correlation between age and concentration of this element (Table 6, Figure 1). In addition to this a significant decrease in Fe and increase in Zn concentration with increasing of age was shown by the Pearson's coefficient of correlation between age and concentration of the elements (Table 6, Figure 1). A change of Br concentration in the prostatic fluid with age from 18 to 82 years is more ideally fitted by a logarithmic law, Fe and Rb - by a linear law, Sr - by an exponential law, and Zn by a polynomial law (Fig. 1). In our study the best fit in the proportion variance accounted for (i.e. R^2) sense maximizes the value of R^2 using a linear, polynomial, exponential, logarithmic or power law. As per author's current information, no published data referring to age-related changes of trace element concentration in human prostatic fluid is available with the exception of Zn. Our finding for the Zn age-dependence does not agree with published data. For example, in the first quantitative X-ray fluorescent analysis of Zn concentration in prostatic fluid of 8 apparently healthy men aged 25-55 years no significant variation with age was recognized [53]. However, no any statistical treatment of results was done in this investigation. Using Atomic Absorption Spectrophotometry (AAS) for Zn measurement in prostatic fluid specimens obtained from 63 normal male subjects in age from 24 to 76 years Fair and Cordonnier [54] did not find any changes in metal level with age.The conclusion was followed from the level of differences between the mean Zn results for three age groups evaluated by parametric Student's *t*-test. Additionally, Zn, concentration in prostatic fluid showed no age relationship in the study of Kavanagh et al. [55] when 33 specimens obtained from normal male subjects in age from 15 to 85 years were measured by AAS and the Pearson correlation between age and Zn concentration was used.

The data of inter-correlation calculations (values of *r* - coefficient of correlation) including all trace elements identified by us in the normal prostatic fluid of males aged 18-40, 41-82, and 18-82 years are presented in Table 7. A significant direct correlation, for example, between the Br and Sr, Fe and Rb, Fe and Sr, Sr and Zn concentrations was seen in prostatic fluid of male of the first age group (Table 7). In age group 2 many correlations between trace elements in prostatic fluid found in the age group 1 are no longer evident (Table 7). For example, correlations between Br and Sr, Fe and Rb, Fe and Sr, Sr and Zn, existed in the age from 18 to 40 years, disappeared but new direct Br-Fe and inverse correlation Br-Zn were arisen.

Thus, if we accept the levels and relationships of trace element concentrations in normal prostatic fluid of males in the age range 18 to 40 years as a norm, we must conclude that after age 40 years the level of Br, Fe and Zn, as well as relationships of trace element concentrations in normal prostatic fluid significantly changed.

No published data on inter-correlations of Br, Fe, Rb, Sr, and Znconcentrations in normal prostatic fluid and age-related changes of these inter-correlations was found.

The ^{109}Cd radionuclide-induced EDXRF analysis developed to determine the Br, Fe, Rb, Sr, and Znconcentrations in prostatic fluid samples is a nondestructive method. It has a great advantage over destructive analytical methods. Almost all analytical methods used for chemical element measurements in prostatic fluid were based on investigation of processed fluid with a goal to destroy and remove organic matrix. In such studies prostatic fluid samples were acid digested or dried under high temperature before analysis. There is evidence that certain quantities of trace elements are lost as a result of such treatment [56-58]. Thus, when using destructive analytical methods it is necessary to control for the losses of trace elements, for complete acid digestion of the sample, and for the contaminations by trace elements during sample decomposition, which needs adding some chemicals. It is possible to avoid these not easy procedures using non-destructive methods, including the ^{109}Cd radionuclide-induced EDXRF.

The ^{109}Cd radionuclide-induced EDXRF developed to determine trace element concentrations in prostatic fluid is micro method because sample volume 20 μl (one drop) is quite enough for analysis. It is another advantage of the method. Amount of human prostatic fluid collected by massage of the normal prostate is usually in range 100-500 μl [59, 60] but in a pathological state of gland, particularly after malignant transformation, this amount may be significantly lower. Therefore, the micro method of ^{109}Cd radionuclide-induced EDXRF developed to determine trace element concentrations in prostatic fluid is available for using in clinical studies.

CONCLUSION

The facility and method for ^{109}Cd radionuclide-induced EDXRF were developed to determine the Br, Fe, Rb, Sr, and Zn concentrations in the micro samples (20 μl) of expressed prostatic fluid. The results of trace element analysis in the micro samples are sufficiently representative for assessment of the Br, Fe, Rb, and Zn concentration in the prostatic fluid.

The means of Zn and Rb concentration obtained for prostatic fluid agree well with median of reported means. For the first time the Fe, Br, and Sr concentrations were determined in the human prostatic fluid, as well as an age-related increase in Zn and decrease in Br and Fe concentration was observed. Moreover, a disturbance of intra-trace element relationships with increasing age was found. Thus, the data does support our hypothesis about involvement of age-related changes of trace element concentrations and their relationships in prostatic fluid in etiology and/or pathogenesis of prostate diseases.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between two authors. VZ collected prostatic fluid samples, designed the EDXRF of samples, and carried out the statistical analysis of results. SZ managed the literature searches, wrote the first draft of the manuscript, and translated the manuscript into English. Both authors read and approved the final manuscript.

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RESEARCH ARTICLE

Assessment of post intervention of geohelminth infection and risk factors among school aged children in the most endemic area of Kano, Nigeria

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Ethical considerations: Introductory letter was sought and collected from Department of Biological Sciences. This was submitted to the State Universal Basic Education Board (SUBEB) for approval of the study. Clearance letter collected from SUBEB (Ref no. SUBEB/POL/138) was used as an introductory letter which was shown to Education secretary of the Local Government as well as principals and teachers of the schools.

ABSTRACT

This study is an initial attempt at determining the prevalence of geohelminth infections among school aged children (SAC) in the most endemic area of Kano State as well as risk factors associated with the infection and the impact of deworming programme in SAC. A retrospective study on the prevalence of geohelminth infection in the 44 Local Government Areas (LGA) of Kano State was conducted. A stratified random sampling technique was used for sample collection. A total of 3000 children were recruited aged 6-15 years. Retrospective study showed that none was of high endemicity. The present status of geohelminth showed that only hookworm was present among SAC with a prevalence of 2.2% and intensity was light (mean: 17 epg). Risk factors that predispose SAC to geohelminth infection like eating outside home, poor hand washing practice, and nail biting were found not to be significantly associated with hookworm infection except risk factor like walking bare footed which was significantly associated with hookworm infection. In conclusion, Prevalence of geohelminth infection in Kabo LGA prior to deworming was 35.1% and post intervention among SAC was generally low (2.2%). The observed low prevalence of geohelminth infection could be attributed to the success of the deworming programme carried out in the district in 2013. Risk factors like poor hand washing practice (2.3%), walking bare footed (2.6%) and eating outside home (2.6%) were pre-dominant among SAC.

Keywords: Geohelminth; Hookworm; Retrospective; Risk factor; Deworming; Post intervention.

INTRODUCTION

Geohelminths are a group of intestinal parasites belonging to the class Nematoda and are transmitted primarily through contaminated soil [1]. The most prevalent geohelminths are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) [2], each parasitizing hundreds of millions of people [1, 3]. Geohelminth infections are common in tropical and subtropical regions of the developing world especially in Sub-Saharan Africa (SSA), where poor domestic and environmental hygiene prevails [4]. More than 1.2 billion people are infected with *Ascaris*

lumbricoides; 740 million people with hookworm; 795 million with *Trichuris trichiura* and 300 million with enterobiasis [5, 6]. Nigeria, the most populous country in SSA, is endemic for geohelminth infections due to ascariasis, trichuriasis, and hookworm with estimated cases of 55 million, 34 million, and 38 million, respectively [7-9]. Favourable edaphic and climatic conditions contribute to the development of the geohelminth infection, while inadequate sanitation facilities, lack of safe drinking water source, poor nutrition, and overcrowding are factors aiding their transmission [10, 11]. Infection may be direct or indirect through secondary sources such as food, water, vegetables and fruits since most geohelminth infections are acquired through the faecal-oral route. Ihesiulor et al. [12] found out in their study that, there was repeatedly moderate prevalence of geohelminth infection among apparently healthy children in Kano Municipal.

Like any public health intervention, however, deworming for geohelminth infections must be justified by evidence and judiciously implemented, especially when very young children are targeted for treatment. From the health perspective, there is now ample evidence clearly demonstrating that regular treatment of geohelminth infections produces immediate as well as long-term benefits, significantly contributing to the development of affected individuals, particularly children [13-15]. Geohelminth treatment is also one of the key components of the preventive chemotherapy package concept [16]. School based de-worming has been recommended as a highly cost-effective public health measure in less developed countries [17]. The World Health Organization (WHO) also recommends a baseline survey in school children to determine the prevalence and intensity of infections [13], and develop effective treatment strategies and case management options [18]. Various school-based surveys have been carried out in Nigeria to estimate the current status of geohelminth infections [19-23]. This study therefore aimed at determining the prevalence of geohelminth infections among school aged children in the most endemic areas of Kano State and the impact of deworming programme in school age children.

MATERIAL AND METHODS

Study area

Kano State is located in North Western Nigeria with latitude 11°30′N 8°30′E and longitude 11.500°N 8.500°E. Out of the 44 Local Government Areas (L.G.A) in Kano State, 17 L.G.A. were reported to be endemic for geohelminth infection following the survey conducted by Kano State Ministry of Health (KMoH, 2013). These are: Tofa (24.8%), Bagwai (20.6%), Kabo (35.1%), Kunchi (25.7%), Shanono (23.5%), Kura (24.6%), Madobi (25.3%), Bunkure (22.9%), Rogo (24.3%), Sumaila (20.4%), Takai (20.6%), Karaye (21.8%), Kiru (20%), Rimin Gado (23.4%), Gezawa (30%), Warawa (28.2%), Gabasawa (26%) L.G.As. School aged children study was conducted in the most endemic area (Kabo 35.1%).

Assessment of impact of deworming program among school aged children

The study population was school aged children (6-15 years) in the most endemic area who were present during the study period. The level of geohelminth infection was assessed. The prevalence of geohelminth infection was compared with the prevalence result obtained by the Kano State Ministry of Health.

Retrospective study of geohelminth infection in forty four local governments

Data on the prevalence of geohelminth infection in the forty four (44) local governments was obtained from department of Neglected Tropical Diseases, Kano State Ministry of Health from a pre intervention study conducted in 2011. It was a cross sectional study involving both sexes. Prevalence was compiled and analyzed using simple mean and percentage to obtain prevalence rate for each local government. According to WHO (2012), geohelminth infection endemic areas are classified into three categories in line with application of MDA: i) high transmission (where prevalence is > 50%), ii) moderate transmission (where prevalence is

between 20-50%), and iii) low transmission (where prevalence is < 20%). Kano State was categorized base on the criteria using a colour coded map to show level of endemicity.

Ethical clearance

Introductory letter was sought and collected from Department of Biological Sciences. This was submitted to the State Universal Basic Education Board (SUBEB) for approval of the study. Clearance letter collected from SUBEB (Ref no. SUBEB/POL/138) was used as an introductory letter which was shown to Education secretary of the Local Government as well as principals and teachers of the schools.

Determination of prevalence and intensity of geohelminth infection in school aged children

Sample size that was used for the school aged children was 3000. This was obtained using stratified random sampling technique with LGA, WARDS, SCHOOLS and CLASSES used as strata. One (1) L.G.A (the most endemic area) was selected. Five Wards were randomly selected from this L.G.A., while 2 schools were randomly selected from each ward. Lastly, 50 children were selected randomly from each class across classes 1-6 to give a total of 1x 5 x 2x 50 x 6= 3000.

Sample collection in school aged children

Following parental/guardian consent, a labeled vial bottle with a tight fitting lid, an applicator stick and a piece of paper were given to them. They were asked to collect fresh stool sample on a piece of paper and an applicator stick should be used to transfer the specimen into the container. The specimens were examined using formalin-ethyl acetate concentration for presence of parasite eggs in the stool [24].

Stool analysis

About 10 ml of 10% formalin was added to 1 g of feaces and stirred using an applicator stick until a slight cloudy suspension was formed. A gauze filter was fitted into a funnel and placed on top of the centrifuge tube. The faecal suspension was passed through the filter into the centrifuge tube until a mark of 7 ml was reached. The filter was removed and discarded with the lumpy residue. 3 ml of ethyl acetate was added to the faecal suspension and mixed for a minute. The centrifuge tube was transferred into the centrifuge and run for 1 minute at 750-1000 g (approximately 3000 rpm). The fatty plug (debris) was loosened with an applicator stick and the supernatant was poured away quickly by inverting the tube. The tube was placed in its rack to allow all the fluid on the sides of the tube to drain down to the sediment. The sediment was stirred and a drop was transferred to the microscopic slide for examination. The whole area of the sediment was examined using x10 and x40 objectives for ova and larvae. Intensity of the infection was estimated based on number of eggs in 1g of stool i.e. egg per gram (EPG) and it was categorized into light, moderate and heavy [25]. Light intensity infection for *T. trichiura* category was defined as 1-999 EPG and the moderate to heavy intensity infection category was defined as ≥1,000 EPG. For ascariasis, light intensity infection was defined as 1-4,999 EPG and moderate to heavy intensity infection was defined as ≥5,000 EPG. For hookworm, light intensity infection was defined as 1-1,999 EPG and moderate to heavy intensity infection was defined as ≥2,000 EPG. The number of EPG of feaces for each species was recorded.

Determination of risk factors associated with geohelminth infection among school age children

Data were collected using a standardized questionnaire. The questionnaire had 9 questions developed in English which was translated to Hausa versions. It was designed to obtain information on risk factors associated with geohelminth infection. School aged children whose parent/guardian must have signed the

consent form were interviewed to obtain information on demographic characteristics and social indicators such as source of water, type of toilet, sanitation, feeding behavior, and type of household were obtained in the questionnaire.

Data analysis

Descriptive statistics was used to analyze the prevalence of the geohelminth infections. Odd ratio was used to test association between the prevalence of geohelminth and risk factors. SPSS version 25.0 was used for analysis of all data and a probability level $P<0.05$ was used to test for significance.

RESULTS

Pre-intervention prevalence of geohelminth infection in 44 local government of Kano State

The prevalence of geohelminth infection in the 44 LGAs of Kano State is presented in Figure 1 in descending order. The highest prevalence rate (35.1%) was recorded in Kabo LGA and the lowest (6.4%) was recorded in Bebeji LGA. Figure 2 illustrate the level of endemicity of geohelminth in Kano State as at 2013. Of 44 LGAs, none was of high endemicity (>50%). Majority (27 LGAs) fell under low endemicity (<20%) with only 17 LGAs being of moderate endemicity (20-50%).

Post-intervention of prevalence of geohelminth infection in school aged children

Table 1 summarizes the demographic characteristics of School Aged Children (SAC) surveyed. A total of 3000 SAC from Kabo Model Primary School, Kabo Central Primary School, Garo Central Primary School, Abdun Garo Primary School, Gammo Central Primary School, Gwaraji Central Primary School, Gude Central Primary School, Mahuta Central Primary School, Godiya Central Primary School and Balan Central Primary School were sampled in the study. Out of the 3000 SAC who were enrolled in this study, 1568 (52.3%) were males and 1432 (47.7%) were females. The mean age of SAC was 10.4. The age range was 6-15 years. Sampled SAC were from classes one to six. Majority of the parent/guardian (71.9%) were not educated and were farmers (59.8%).

Table 2 shows the prevalence rates of geohelminth infection in SAC in Kabo Local Government Area (LGA), 3 years after the deworming programme. Out of 3000 School Aged Children examined, 66 (2.2%) were infected with geohelminths. The only helminth encountered was hookworm. Rate of infection varied across schools ranging from 0.7% in Mahuta Central Primary School to 3.7% in Balan Central Primary School. The prevalence of geohelminth infection in male and female children was 1.3% and 0.9% respectively. The intensity of infection was characterized based on the WHO grouping system of geohelminth infection intensities [13]. All the children had light intensity of geohelminth infection (mean: 17 epg).

A comparison between the findings of the geohelminth infection assessment reported in the present study with those of the reported data of the study carried out in 2013 by the KSMoH as shown in table 3 reveals a drastic reduction in the prevalence rate from 35.1% to 2.2% in Kabo L.G.A of Kano State.

Risk factors associated with geohelminth infection among school aged children

Findings on the assessed risk factors associated with geohelminth infection are prevalent among SAC as shown in Table 4. The result show that, inspite of the low prevalence of infection risk factors associated with geohelminth infection are highly prevalent among the children.

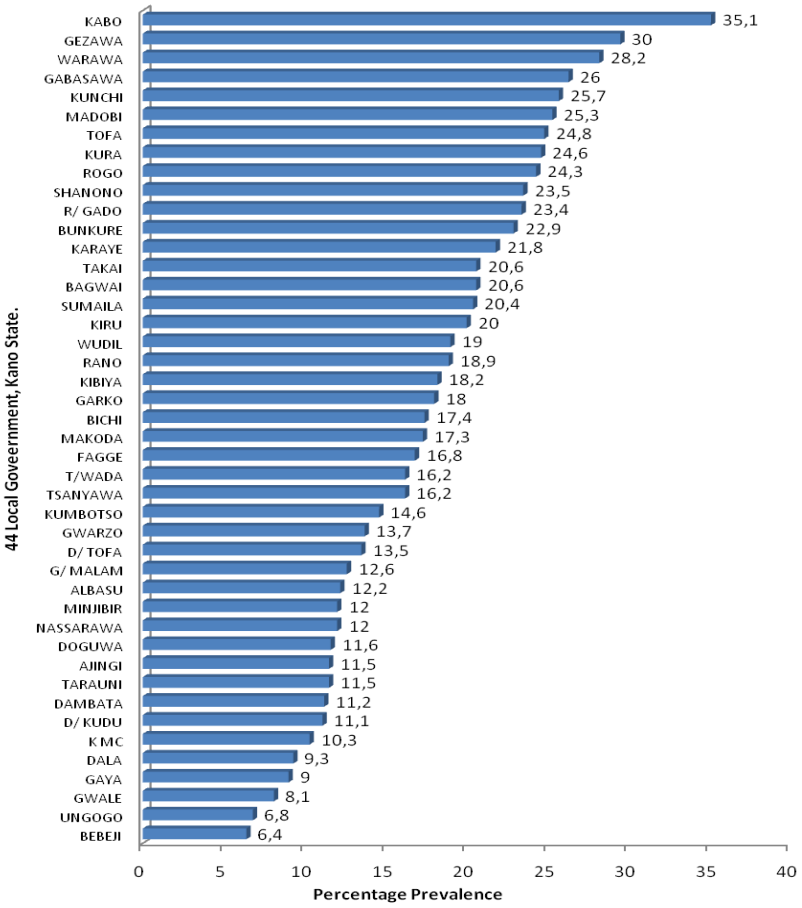

Figure 1. Prevalence of geohelminth infection in descending order in the 44 local government of Kano State.

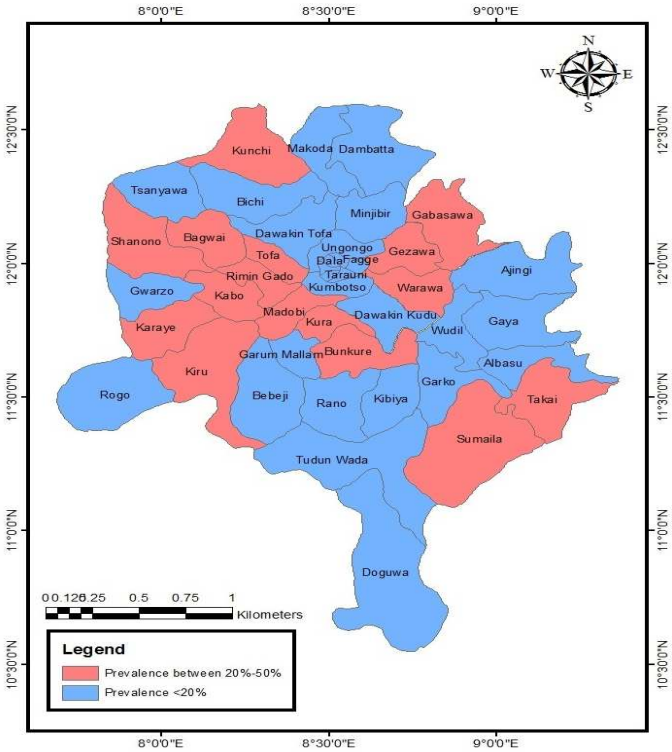

Figure 2. Map of Kano State showing endemicity of geohelminth infection.
Key: High transmission: prevalence >50%. Moderate transmission: prevalence between 20-50%. Low transmission: prevalence <20%.

Factors such as poor housing quality (mud houses) and poor toilet facilities (pit latrines) were recorded for all the participants. Majority of the children walk bare footed (63.7%) and eat outside home (62.7%). Many do not wash hands before meals (46.7%) nor after use of the toilet (46.7%) while an appreciable proportion practice nail biting (26.3%).

Analysis of association of these risk factors with geohelminth infection among the SAC revealed that only two of the assessed risk factors were significantly associated with infection among the SACs. The odds of having infection was almost 2 times higher among participants who walked barefooted than in those who wore shoes (OR=1.8, p=0.04).

Similarly, the odds of being infected was about twice higher among those who ate home cooked meals (OR=1.73, p=0.051). Although only two factors were found to be significantly associated with geohelminth infection in this study, yet infection rate was high among individual where the risk factor were prevalent.

Table 1. Demographic characteristics of school aged children.

Variable	Total (%)
Age group (years)	
6-10	1617 (53.9)
11-15	1383 (46.1)
Total	3000
Gender	
Male	1568 (52.3)
Female	1432 (47.7)
Total	3000
Parent/Guardian education	
Not educated	2156 (71.9)
Educated	844 (28.1)
Total	3000
Parent/Guardian occupation	
Farmer	1794 (59.8)
Trader	370 (12.3)
Civil service	696 (23.2)
Others	140 (4.7)
Total	3000

Table 2. Prevalence of geohelminth infection among school aged children.

Schools	No. examined	Hookworm		Total infected (%)	Mean egg per gram
		Male (%)	Female (%)		
Kabo Model Primary	300	4(1.3)	2(0.7)	6 (2.0)	2
Kabo Central Primary	300	4(1.3)	4(1.3)	8 (2.7)	1
Garo Central Primary	300	6(2)	4(1.3)	10(3.3)	2
Abdun Garo Primary	300	7(2.3)	2(0.7)	9 (3.0)	1
Gammo Central Primary	300	3(1)	0(0)	3 (1)	2
Gwaraji Central Primary	300	2(0.7)	4(1.3)	6 (2)	1
Gude Central Primary	300	4(1.3)	3(1)	7 (2.3)	2
Mahuta Central Primary	300	2(0.7)	0(0)	2 (0.7)	1
Godiya Central Primary	300	4(1.3)	0(0)	4 (1.3)	2
Balan Central Primary	300	4(1.3)	7(2.3)	11 (3.7)	1
Overall	3000	40 (1.3)	26(0.9)	66 (2.2)	17

Table 3. Comparison of data on geohelminth infection in 2013 with data of present study.

Source of information	No. examined	No. infected	% Prevalence
Data from 2013 KSMoH surveyed in Kabo LGA Kano	248	87	35.1
Present surveyed SAC in Kabo LGA Kano	3000	66	2.2

KSMoH - Kano State Ministry of Health, SAC - School Aged Children, LGA - Local Government Area.

Table 4. Risk factors associated with geohelminth infections among school aged children.

Variables	Categories	Frequency n=3000	No. infected (%)	OR	p-value
Eat outside home	Yes	1182	49 (2.6)	1.731	0.051
	No	1118	17 (1.5)		
Do not wash hands before meal and after toilet	Yes	1401	32 (2.3)	0.929	0.769
	No	1599	34 (2.1)		
Do not wear shoes	Yes	1912	50 (2.6)	1.80	0.040
	No	1088	16 (1.5)		
Nail biting	Yes	790	15(1.9)	0.819	0.501
	No	2210	51(2.3)		

OR - Odd Ratio.

DISCUSSION

With regards to mapping of geohelminth infection in Kano State, this study has provided data on the prevalence of geohelminth infection generated from the 44 LGAs of Kano State. The data has been presented in a bar chart (Figure 1) which reflects the level of endemicity in the different local government areas. Endemicity is a measure of disease prevalence in a particular region, while prevalence is the proportion of the people infected at a given point in time. According to WHO, geohelminth infection endemic areas are classified into three categories in line with application of MDA: i) high transmission (where prevalence is >50%), ii) moderate transmission (where prevalence is between 20-50%), and iii) low transmission (where prevalence is <20%) [26]. In the present study, no LGA was above 35%, placing the area in moderate to low transmission zone.

The prevalence of geohelminth infection among school aged children (SAC) was 2.2%. According to WHO [27], if 66 positive children are found, the area is classified as being in the soil transmitted helminth prevalence range of 20% to < 50%. There is reduction in the prevalence of geohelminth infection in the study area compared to the pre-intervention prevalence rate of geohelminth infection (35.1%) recorded by Kano State Ministry of Health (KSMoH) [28]. Reduction in the prevalence was due to the deworming intervention programme taking place in the study area which was distributed by Health and Development Support (HANDS) programme and Christian Blindness Mission (CBM) to the Ministry of health. This shows that the anthelminthic drugs were effective in reducing the prevalence of geohelminth infection. This study is in accordance with a study done by [29] in Kwazulu-Natal South Africa which showed that single dose treatment with albendazole was very effective against hookworm and *A. lumbricoides* with cure rates (CR) of 78.8% and 96.4% and egg reduction rates (ERR) of 93.2% and 97.7%, respectively however it was exceptionally ineffective against *T. trichiura* (CR = 12.7%, ERR = 24.8%). Also, the study is in accordance with a study done by [30] in Nigeria suggested that at baseline, the number of moderate infections was 6.2% and by the end of the follow-up after administration of albendazole the number of moderate infections dropped to 1%. [31-33] supported the use of albendazole for mass chemotherapy because of its effectiveness. School-based deworming also has major externalities for untreated children and the whole community by reducing disease transmission in the community as a whole [34]. Treatment with anthelminthic drugs reduces the transmissibility of the

parasite by reducing worm load and shedding of eggs [35], with a single dose of anthelminthics resulting in CR of 88% for *A. lumbricoides* and 78% for hookworm [36]. A study on the efficacy of a mass drug administration programme from South India revealed that periodically administering albendazole reduced the geohelminth infection burden by 77% [37]. On visual examination, all schools visited had pit latrines and majority of the SAC wore shoes. This could partly explain for the observed low prevalence of geohelminth infection among SAC. Prevalence of geohelminth infection in male SAC (1.3%) was an indication that special activities of males such as walking in farm, playing football, walking in flood after rain fall, playing in contaminated soils and molding houses using moist soils could have predisposed them to infections [38]. Sometimes these activities are carried out in the study area while they are bare footed. This was supported by previous studies in Nigeria and India [20, 23, 39, 40] who separately reported high prevalence of geohelminth infection parasites among males than females due to their activities.). Number of eggs per gram counted showed that geohelminth infection (hookworm) was categorized as light. The relatively low prevalence value could be attributed to the inability of the 3rd stage infective larvae to access human skin as penetration of skin which is the major route of infection [41]. Another explanation for the relative low prevalence could be attributed to the survival rate of the larvae in the soil as texture and type of soil markedly influence the viability of the 3rd stage infective larvae [42]. The viability of the larvae is optimal on sandy, warm, humid soil [43].

While low-parasitic burden in a community is an indication of endemicity and chronicity [44, 45], from the public health point of view it is often interpreted as low health impact and therefore low priority [46, 47]. Moreover, in communities where deworming programs are implemented, low intensity infections might be interpreted as a success indicator [48, 49]. The absence of moderate and heavy intensity of infection could be the result of the mass chemotherapy which was done in 2013 [28] and probably some other behavioral and environmental factors that discourage transmission of geohelminth infection among SAC in the district.

A direct comparison of the survey from 2013 with the data of the present study could not be carried out fully because: the 2013 survey was community based while the present study is school based. Secondly, the ages of the children in 2013 survey were not available.

Among associated risk factors, eating outside home (street vended food), poor hand washing habit before meal and after toilet, not wearing shoe and nail biting were the major factor among SAC in the study area; and they were found not to be significantly associated ($p>0.05$) with geohelminth infections except risk factor such as not wearing of shoe and eating outside home (street vended food), which was significantly associated ($p<0.05$) with geohelminth infection. Bearing in mind that only hookworm was detected in this study, this present finding on the risk factors may be connected with the fact that hookworms are transmitted majorly via skin penetration. Therefore not wearing of shoe is a major factor in this instance. All the respondents mentioned mud bricks and pit latrine as the quality of housing and toilet facility both of which provides conducive environments for the geohelminth infection. This finding is accordance with a study conducted in Turkey by [50] who reported that children living in shanty areas had a higher risk of geohelminth infection than those living in towns. Usage of pit latrines in this study were the commonly used sites of sewage disposal which is in accordance with a study conducted by [11] who stated that the use of pit latrine reflects the poor socioeconomic status of the study subjects. Sufiyan et al. [51] in Nigeria concluded that participatory hygiene education to deworming programmes will greatly improve the hemoglobin level of children in areas where there is a high prevalence of hookworm infections. The appropriate mix of interventions for responding to geohelminth infection globally include access to safe water and provision of effective sanitation facilities will help to break the helminth transmission cycle; skills-based education, including life skills that address health and hygiene issues and promotion of positive behaviors; simple, safe, and familiar health and nutrition services that can be delivered cost-effectively in schools (such as deworming).

CONCLUSION

Pre-intervention survey revealed that none was of high endemicity. The highest prevalence (35.1%) was recorded in Kabo LGA and the lowest (6.4%) was recorded in Bebeji LGA. Present status of geohelminth

infection in SAC shows an overall prevalence of 2.2%. The drop in the rate of infection from 35.1% to 2.2% (in Kabo) indicates a marked improvement in the health status of SAC in the State. Risk factors that predispose SAC to geohelminth infection like poor hand washing practice (2.3%), walking bare footed (2.6%), eating outside home (2.6%) was pre-dominant among SAC.

AUTHORS' CONTRIBUTIONS

TIO contributed in the conceptualization, data analysis, drafting and proof reading of the manuscript. ZG contributed in the data collection, writing of the manuscript, analysis of the data and final draft of the manuscript. BB was involved in the data collection, statistical analysis and proof reading of the manuscript. All authors read and approved the final manuscript.

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